CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(*formerly* Scientific Agriculture)

VOLUME 36 MAY-JUNE 1956 No. 3

CHROMOSOME BEHAVIOUR AND FERTILITY OF TETRA PETKUS RYE[1]

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[Received for publication October 14, 1955]

ABSTRACT

The analysis of the configurations present at first metaphase shows that there is no difference between strains of Tetra Petkus nor between them and other autotetraploid rye varieties. Any increase in fertility (seed setting) is not accompanied by a change in meiotic behaviour. Selection for reduced multivalent formation and consequently higher fertility is only a slim possibility. About 15 per cent of the plants in a population of autotetraploid rye are aneuploids with chromosome numbers of 27, 29 and 30. These aneuploids have lower seed setting than those plants with 28 chromosomes. Fertility in diploid varieties of rye exceeds tetraploids by 10 per cent.

INTRODUCTION

"Tetra Petkus" is an autotetraploid rye (2n = 28) which was produced by doubling the chromosome number of the original diploid strain with colchicine. It was produced in Germany by W. Laube, of F. von Lochow Petkus Ltd. In August 1954, a licence certificate was issued to cover the sale of "Tetra Petkus" in Canada, based on the recommendation of the Ontario Agricultural College, Guelph. Tests showed that it had great vigour, stiff straw and strong roots, and that it outyielded a standard diploid variety.

"Tetra Petkus" originated about 15 years ago and since then has undergone considerable selection, chiefly for increased fertility (8). Rye is a cross-pollinated crop and the production of self-fertile lines of diploid stocks has invariably led to lower yields. There is a similar drastic reduction in seed set when tetraploid rye is selfed (5). Any increased fertility in tetraploid rye is probably gene-controlled, although some workers have tried to obtain increased stability and higher fertility by selecting for a *regular meiosis* (7). Not all agronomists agree that this is possible. From the studies of many cytologists it is known that the chromosomes of an autotetraploid do not only form bivalents at meiosis. Because of the duplication of the chromosomes, quadrivalents, or trivalents and univalents, are common. The eventual distribution of the chromo-

[1] Contribution No. 197, Cereal Crops Division, Experimental Farms Service.

[*The March-April number of this Journal* (*Volume 36, No. 2*) *was issued April 30, 1956*].

somes to the pollen grains and egg-cells gives irregular numbers which cause aneuploid progeny and may cause sterility. The problem of correlating cytological behaviour with seed set is, therefore, an ideal one for a cytogeneticist. The recent introduction of "Tetra Petkus" into Canada makes it timely.

MATERIAL AND METHODS

Two lots of tetraploid rye were sent to the Cereal Crops Division by the Agricultural and Industrial Development Corporation, Harrisburg, Pennsylvania. One lot was received in October 1951, and hereafter is called "T.P. 51"; the other sample came in 1954—"T.P. 54". Under the system for maintaining or improving Tetra Petkus, new stocks are released each year.

For cytological studies whole spikes were fixed in Carnoy's (6 : 3 : 1). All meiotic studies were made from acetocarmine squashes of anthers. The cytological data in T.P. 51 were taken from 7 plants. In T.P. 54 meiosis was studied in 112 plants; the data on metaphase were compiled from 4 plants after proving that there were no significant differences between them. The 112 plants were selected at random from another group of 250 plants that had been sampled from an isolation plot. Field samples were taken on 5 different days to obtain a range in plant maturity.

In estimating the amount of seed set, the top and bottom spikelets were not scored. Forty-eight florets were counted in long spikes and 28 in short spikes. In other words, estimates of fertility were based on the spikelets in the middle portion of the spike. When spikes were bagged for fertility studies, the bags were shaken at least once a day over the critical 3-day period.

RESULTS

Meiosis in Autotetraploid Rye

The behaviour of the chromosomes of tetraploid rye at meiosis is outlined in Figure 1. The chart was not designed to follow meiosis in any one particular cell, but to give a survey of typical behaviour at several stages. Thus, in the type of cell shown at metaphase, the chromosomes would probably divide regularly and would not produce univalents that would misdivide or form micronuclei. Other cells with different configura-

TABLE 1.—FREQUENCY OF QUADRIVALENTS AT METAPHASE I IN PLANTS WITH 28 CHROMOSOMES

Variety	No. of cells	No. of quadrivalents[1]	
		Range	Mean
Steel-rye Muntzing (5)	58	1–7	3·9
Tetra Petkus, 1951	100	1–7	4·2
Tetra Petkus, 1954	100	1–6	3·9

[1] Includes some trivalents.

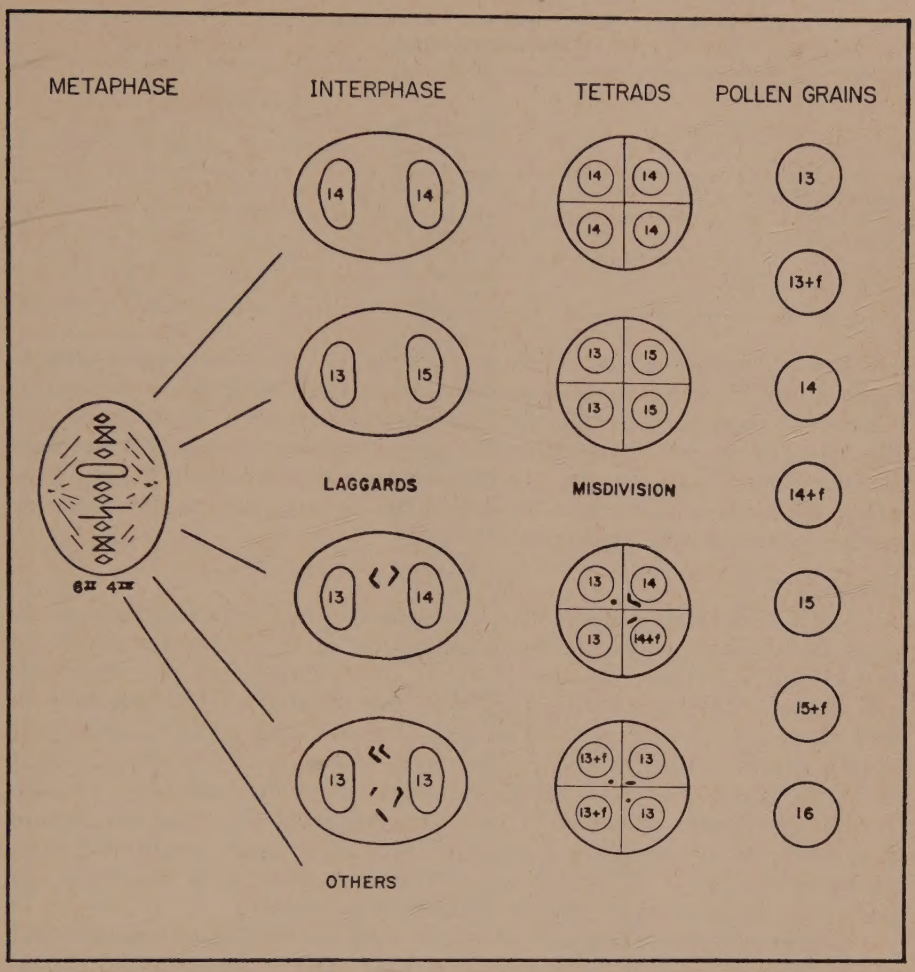

FIGURE 1. The behaviour at meiosis of the chromosomes of autotetraploid rye.

tions of chromosomes at metaphase 1 could give the various types of interphases, tetrads and pollen grains. Details on most phases are given in the following sections.

Metaphase I

The analysis of the configurations present at first metaphase shows that there is very little difference between varieties or between different strains of Tetra Petkus (Table 1). The quadrivalents were orientated as zig-zags, chains or rings. Crowding of chromosomes and variations of the standard zig-zag type of quadrivalent, that looked somewhat like a "frying-pan" trivalent, made it difficult to separate all associations into specific categories. In T.P. 51 there was a ratio of one zig-zag to one of any other type. The number of trivalents in 28-chromosome plants was surprisingly low. In T.P. 51, 5 per cent and in T.P. 54, 8 per cent of the cells had a

TABLE 2.—CHROMOSOME DISTRIBUTION AT ANAPHASE I IN PLANTS WITH 28 CHROMOSOMES

Variety	No. of cells	Distribution to the poles		
		14-14	13-15	Others
Steel-rye Muntzing (5)	40	16	9	15
Tetra Petkus, 1951	50	43	3	4
Tetra Petkus, 1954	50	34	5	11

trivalent plus a univalent. Only a few cells had unpaired chromosomes. Most of the bivalents were closed (ring) bivalents with three chiasmata. Interstitial chiasmata in quadrivalent associations were prominent. No differences in chiasma frequency nor terminalization were found between the two Tetra Petkus stocks. In hypertetraploid plants the extra chromosomes were either lying free as univalents or attached to bivalents or quadrivalents forming higher associations.*

Anaphase I

When slides were prepared there were only a few cells per slide at an anaphase stage in which a clear distribution of the chromosomes could be made out. In those few cells, however, the chromosomes were unusually well spread. It was at this stage that the number of chromosomes in each plant was determined. The quadrivalents and bivalents disjoined cleanly with few bridges and in most cells there were 14 chromosomes at each pole (Table 2). The difference between Steel-rye and Tetra Petkus, though significant, is probably due to conditions, e.g., the results are from many plants and varied greatly. Muntzing came to a similar conclusion in his work. Other tetraploid varieties examined by Muntzing (5) gave distributions very similar to Tetra Petkus.

Lagging chromosomes (univalents) sometimes divided mitotically and also misdivided. There were more laggards in the hypertetraploid plants.

Tetrads

In plants of T.P. 54 with 28 chromosomes about 20 per cent of the tetrads had one or more micronuclei—different counts ranged from 15–32 per cent. In plants with 29 chromosomes, 50 per cent of the tetrads had micronuclei. Over 200 tetrads for each of 3 plants were examined. The results were so close to 50 per cent in each count that this criterion could possibly be used for a preliminary selection of aneuploids. In plants with 29 chromosomes in Steel-rye [Muntzing (5)], 53 per cent of the tetrads had micronuclei; in plants with 28 chromosomes, 23 per cent had micronuclei.

Pollen Grains

Mature pollen grains of T.P. 54 were compared with those of diploid rye. Tetraploid pollen is larger, but both had about 5 per cent abnormal

* In one slide there were 11 cells with only 14 chromosomes. One cell was at anaphase; in 6 cells at metaphase there were 3^{I}, 4^{II}, 1^{III} and in 4 cells, 2^{I}, 3^{II}, 2^{III}. Aneuploid cells are not uncommon [O'Mara (6), Morrison (4)]. It is interesting to note that in the cells with 14 chromosomes there has been no directed "reductional grouping" as suggested in early theories by Huskins and co-workers (2, 3).

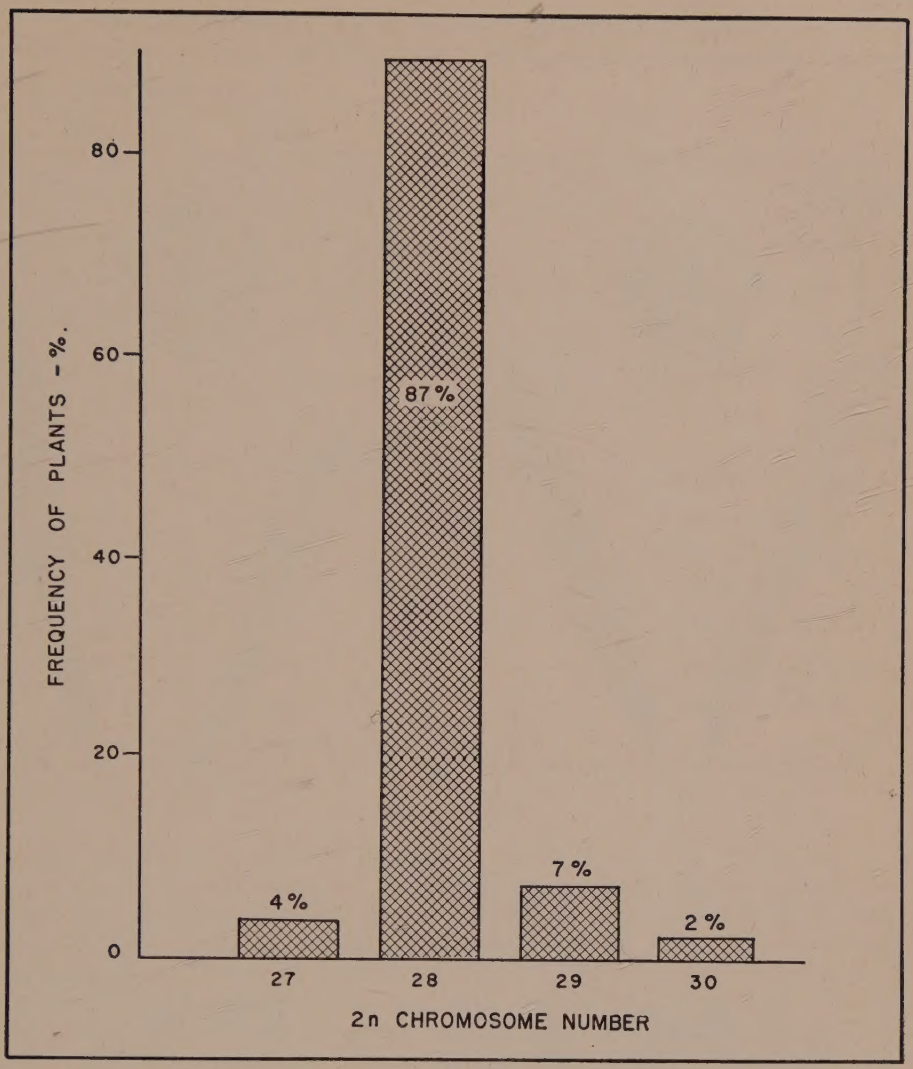

FIGURE 2. Distribution of aneuploids and euploids in 100 plants from a plot of T.P. 54.

or uninucleate pollen grains. Muntzing (5) claims that the majority of tetraploid plants have 90-100 per cent good pollen grains.

Chromosome Numbers and Fertility in T.P. 54

One hundred plants were picked at random and the number of chromosomes in each plant was determined (Figure 2.) In another smaller sample of 12, there were two aneuploids, both with 29 chromosomes.

Seed set was determined in spikes selected at random, in spikes with a known complement of 28, in the aneuploids and in some diploid strains of rye as checks (Table 3.) The amount of seed setting varied from one plant to another and also varied between different tillers on the same plant (Figure 3).

TABLE 3.—THE FERTILITY OF DIPLOID AND AUTOTETRAPLOID RYE

Variety	Fertility in per cent	
	Range[1]	Mean
Diploids		
Dominant	71–98	86·7
Imperial	58–96	89·5
Petkus	81–94	88·5
Steel-rye Muntzing (5)	—	78·5
Tetraploids		
Steel-rye Muntzing (5)	—	62·0
T.P. 54 (2n = 28)	68–94	81·3
T.P. 54 (2n = 28) bagged	7–44	23·2
T.P. 54 (2n = 27)	43–78	62·2
T.P. 54 (2n = 29)	47–78	69·1
T.P. 54 (2n = 30)	65–73	70·4

[1] For T.P. 54 (2n = 30) only 5 spikes were examined; for all others, at least 10 spikes.

DISCUSSION

According to Muntzing (5) tetraploid Steel-rye had been rigidly selected for fertility for 7 years. However, the mean number of quadrivalents in Steel-rye did not differ from that of his other unselected varieties which were of recent origin. There is no difference between T.P. 51 and T.P. 54 nor between these two and Steel-rye. It should be emphasized that all these stocks are different in age, i.e., generations from the time that doubling occurred. Selection for increased fertility has been made in "Tetra Petkus" since it was first produced. It is quite obvious, then, that the selection has not been accompanied by any change in pairing behaviour—as measured by the associations visible at first metaphase. The differences in chromosome behaviour at other meiotic stages are also very small and uphold the conclusion reached above. Any increase in fertility that these stocks possess over the original must have a genetical basis or some obscure physiological cause as outlined by Ramanujam and Parthasarathy (9).

What is the possibility of reversing this procedure and selecting plants that have a regular meiosis, thus achieving higher fertility? While eventually, after rigid selection, there may be an autotetraploid which would behave as an allotetraploid, the process appears likely to be a long one. However, in a recent report from the Swedish Seed Association (12), it is claimed that "selection for forms with regular meiosis and consequent

FIGURE 3. A sample of seed and heads in diploid and tetrapoid rye (natural size).

A. Diploid (2n = 14).
B. T.P. 54 (2n = 28). Note the complete fertility in one spike.
C. Metaphase of meiosis in diploid rye, 7^{II}.
D. Metaphase of meiosis in T.P. 54, 2^{I} 5^{II} 4^{IV}.

fertility in tetraploid spring rye has yielded promising results". As it is obviously normal for an autotetraploid to have some quadrivalents, what is needed is an abnormal meiosis with 14 pairs, or else 7 quadrivalents that will always separate equally.

In nearly all cells at metaphase I there is at least one quadrivalent. In order to be certain that a plant had reduced quadrivalent formation it would be necessary to examine many cells and compute a mean value for the plant. An assumption would also have to be made that the anthers chosen would be representative for the whole plant. Actually, we know that the environment greatly affects pairing formation and this would have to be considered (10, 11, *et al.*). Even so, an intensive cytological search may succeed in isolating a cytologically stable strain. There are two facts which should be encouraging: First, rye in the diploid form has many strains that have meiotic irregularities, asynapsis, etc., and these instabilities could be carried to the tetraploid. Second, as rye is a cross-pollinated crop there is always the opportunity for combination of unlike types. It may thus be possible to alter the chromosomes enough to reduce pairing so that only bivalents are formed. However, it is more than likely that any improvement will be based on the selection of genes. From a study of pairing in many plants, including rye, it has been shown that pairing is controlled to a limited extent by a gene or genes. Perhaps some strain can be isolated that will have some genic barrier which will prevent multivalent formation.

In Steel-rye, Muntzing (5) found that 22.7 per cent of the plants were not true autotetraploids but were aneuploids with 27, 29, or 30 chromosomes. Ten generations later, Hagberg (1) found 14.5 per cent aneuploids in 9 lots of seed. This is a weighted mean adjusted to compensate for fraction sizes. The actual mean (which can be computed from Table 3 [Hagberg (1)] is considerably higher and more in line with Muntzing's results. It is also apparent from Hagberg's figures that there is considerable variability. Some lots of seeds had 40 per cent aneuploids.

A comparison with the results for Steel-rye might lead to an assumption that 13 per cent in T.P. 54 (Figure 2) represents a reduction from the original stock. However, the difference is likely due to sampling; Muntzing's original count may be a little high and the present count may be somewhat low. An average of several years' results would probably fix the number of aneuploids at about 15 per cent. While fertility may not be directly related to meiotic behaviour, aneuploids arise directly out of the distribution of unequal numbers of chromosomes to egg-cells and pollen grains and the chance union of these gametes. An indication of the number of aneuploids expected can be taken from the percentage of tetrads having micronuclei. Here is another reason why the difference in the number of aneuploids, found by Muntzing and in this study, is probably not significant. If a difference occurred between Steel-rye and T.P. 54 in the number of tetrads having micronuclei, or a consistent difference in the distribution of chromosomes at first anaphase, then these differences would be reflected in the number of aneuploid plants produced.

The complete elimination of aneuploid plants from a population of tetraploid rye is not possible as long as there is quadrivalent formation,

except in the special condition of controlled disjunction. There will be unequal separation and lagging chromosomes and, therefore, some pollen grains or egg-cells with fewer or more than 14 chromosomes. A cytological check would reveal aneuploid plants and these could be eliminated and thus reduce the percentage of aneuploids but this process would have to be repeated with every generation. There would still be a portion of the plants arising from 28-chromosome plants that would be aneuploids. There are two mechanisms that will reduce the number of aneuploids: 1. The production of gametes that do not have a deficiency or an excess of chromosomes. They could arise from a regular meiosis of all bivalents or equally separating quadrivalents. 2. The abortion of zygotes that have not 28 chromosomes. There is probably some elimination of unbalanced zygotes in present populations, especially in those that have over 3 chromosomes missing. On the assumption that meiosis in the embryo sac is similar to that in the anthers, deficient egg-cells should be formed in 10-20 per cent of the florets. We know that the competition between balanced and unbalanced pollen grains is quite strong because if it were not there would be many more aneuploids. While this competition will tend to lower the number of aneuploids, it will not eliminate them.

Diploid rye plants have more seed set per spike than tetraploid rye. Muntzing (5) found that tetraploid Steel-rye had 62 per cent fertility and diploid 78.5 per cent. Plarre (7) found 71.3 per cent for tetraploids and 84.6 per cent for diploids. Other workers have quoted 60-95 per cent for diploid rye and a somewhat similar range for tetraploids with the tetraploids always lower than the diploids in specific tests. There was a difference of only 10 per cent between T.P. 54 and the 3 diploids checked. It is obvious that the percentage figures arrived at are dependent upon how they were taken, upon the strains tested, and upon conditions of the plot—whether tetraploids were isolated from diploids, the environment, etc. In estimating the amount of seed setting in this study only the middle part of the spike was used. The figures, therefore, may be slightly higher than those of other workers. At Ottawa, in 1955, the weather was favourable for seed setting—a combination of hot dry days with sufficient wind for carrying pollen. In many diploid spikes only one or two florets were sterile and in the tetraploid there were also some spikes with nearly complete fertility (Figure 3). This rating is not just on the central portion but includes basal and tip florets as well.

In the bagged tetraploid spikes the fertility was higher than other workers have found. Shaking and tapping the bags during pollen shedding may have made some difference. It should be remembered, too, that T.P. 54 has been selected for increased seed setting, and while this need not be accompanied by increased self-fertility, it could be related. One thing is clear, there is reduced seed setting in the aneuploid plants. The aneuploids were as vigorous as the euploids and dehiscence occurred at the same time. Therefore, environment has not caused the drop in fertility. Deficient gametes produced by the plants have probably been ineffective. It would be interesting to study the progeny from each type of aneuploid.

An interesting feature of the fertility of both diploids and tetraploids is the variability that exists between plants. There is also consider-

able variability between tillers of the same plant. The environment at the time of dehescence of a particular spike could facilitate or retard seed setting and an estimate taken from this spike would not be general for the whole plant. It seems reasonable to suppose that the fertility of autotetraploids could be increased, or perhaps only maintained at a high level by the selection of plants with high fertility and then intercrossing them. A microscopical examination to eliminate some aneuploids from the breeding stock would also help to keep the fertility levels up. However, there may be differences between tillers on a plant, as well as differences between plants, and this should be taken into consideration when samples of the population are studied.

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OBSERVATIONS ON THE MINERAL METABOLISM OF PULLETS

XI. THE EFFECTS OF PROTRACTED TREATMENT WITH ESTROGEN AND AND WITH ESTROGEN PLUS ANDROGEN ON RETENTION OF CALCIUM BY THE SEXUALLY IMMATURE PULLET[1]

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[Received for publication December 8, 1955]

ABSTRACT

Sexually immature pullets were treated with 0.5 mgm. estradiol benzoate (ODB) per day or with 0.5 mgm. ODB plus 0.4 mgm. testosterone propionate (TST) and their daily retentions of calcium were compared with that of untreated pullets over a period of 28 days. For the untreated birds the daily Ca retention remained relatively constant throughout, though with a slight tendency to decline over the last 18 days. ODB + TST increased the daily Ca retention rapidly to maxima around days 7-9, followed by a slow decline towards the values for the untreated birds. ODB alone failed to elicit any transient increase of Ca retention over the first three or four days, but Ca retention had declined by about day 10 to minimal values below the level for the controls. Ca retention by the pullets treated with ODB subsequently increased again steadily to attain values at 28 days indistinguishable from those of the controls.

INTRODUCTION

It was shown some years ago that treatment of sexually immature pullets with androgen or with various levels of estrogen did not appreciably affect their average daily retention[6] of Ca; when the same levels of estrogen were administered concurrently with androgen, the average daily retention of Ca was increased to a striking extent (1). These results were subsequently confirmed in general, but it was further shown that, for short-term experimental periods of about two weeks, the *average* daily retention of Ca was, in fact, slightly increased either by androgen alone or estrogen alone, although these effects were of a different magnitude altogether to the effect of estrogen plus androgen (2).

A closer study of the data for day-to-day retention (5) revealed that the slight positive effect of androgen alone on average calcium retention was due to a small sustained effect. The slight positive average effect of estrogen alone was due to a transient pronounced positive effect during the first three or four days after the beginning of the hormonal treatment, followed by a decline in retention, so that by the second week the daily retention was not appreciably different from that of the control birds. This transient positive effect of estrogen alone has been confirmed in subsequent experiments where similar birds and treatments were used.

The general trend of the data for Ca retention secured in this previous work suggested the desirability of ascertaining whether or not a more protracted treatment with estrogen alone would depress Ca retention below that of the controls.

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[4] Professor of Agronomy.
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[6] The term "retention" is used throughout this paper to denote "total Ca intake—total Ca in droppings".

It is of interest here to recall that an increased fragility of the bones in association with estrogenization of poultry has been reported from time to time [for references see the review by Lorenz (6)] although an increased fragility is not necessarily a consequence of a decreased Ca content. Alterations of the nature or pattern of skeletal calcification might have a similar gross consequence.

The present paper describes an experiment undertaken in order to study the effects of fairly protracted treatment with estrogen alone on the daily Ca retention of the sexually immature pullet.

EXPERIMENTAL MATERIALS AND METHODS

General

The experimental birds comprised six crossbred pullets [Rhode Island Red × (New Hampshire × Barred Plymouth Rock)] which were 11 weeks of age when first placed under experimental conditions and 15 weeks of age at the end of the experiment. The six birds were carefully selected for uniformity of appearance, condition and liveweight. They were housed individually in cages measuring 2 ft. × 2 ft. × 2 ft. These cages had wire floors and were supported above galvanized trays so as to permit collection of the droppings.

The feed mixture was made up in parts by weight as follows:— corn meal, 25; ground wheat, 27; finely ground oats, 25; wheat shorts, 5; soya bean oil meal, 15; linseed oil meal, 3; common salt, 0.5; disodium phosphate crystals, 2. Six gm. manganese sulphate tetrahydrate, 200,000 units of 'dry' vitamin A, and 10,000 units of 'dry' vitamin D were incorporated per 100 lb. of this mixture. Feeding was arranged with the aim that each pullet should consume similar amounts of feed mixture and of calcium carbonate. The fixed daily allowance of 70 gm. feed mixture for each bird was weighed out in a separate beaker and to each was added 2.00 gm. $CaCO_3$ U.S.P. Just before feeding, the feed allowance and the $CaCO_3$, were moistened slightly and well mixed by hand in the beakers. This method of feeding avoided most of the error that may arise from differential settling of feed ingredients. The birds cleaned up their daily allowance regularly before nightfall, except for pullet No. 1, which from time to time left a few grains for several successive days. When this took place the residue in the beaker was collected next day before the morning feed and analysed for its Ca content. The Ca intake for such days was then ascertained by difference.

Feeding and collection of droppings were done on a rigidly timed schedule (Eastern daylight-saving time), as follows:—

7.45 a.m. Half of daily feed allowance given.
2.00 p.m. Second half of feed allowance given.
9.00 p.m. Lights out.

Distilled water was provided for drinking. Care was taken throughout the experiment to avoid disturbance of the birds.

The 24-hourly droppings were thoroughly mixed to a uniform pasty mass on the trays, with additions of water as necessary. The moist mass was transferred to Pyrex pie dishes and dried overnight at approximately

80°C.-90°C. in a large air-drying cabinet. Next morning the dry, friable mass was milled in a "Mikro-Samplmill"* immediately on removal from the drying cabinet. Each powder was spread in a Pyrex dish and left to equilibrate with the laboratory air until the following morning, when it was weighed to the nearest 0.05 gm. and bottled pending analysis. This method avoids errors that may be incurred through the hygroscopicity of the dried droppings if the samples are weighed and bottled immediately after grinding.

Analytical Methods

Determinations of Ca in food and dried droppings were carried out on the macro scale by ashing followed by precipitation of Ca as the oxalate and titration with standard permanganate. Serum calcium was determined on a trichloracetic acid serum filtrate by titration with ethylenediaminetetracetic acid using a murexide indicator (4).

Experimental Treatments

After the birds had been given a week to become used to the experimental conditions, they were placed on calcium balance and given the following daily dosages of gonadal hormones by intramuscular injection:

Pullets No. 1 & 4 Nil
Pullets No. 2 & 5 0.5 mgm. estradiol benzoate (ODB)†
Pullets No. 3 & 6 0.5 mgm. ODB plus 0.4 mgm. testosterone (TST)‡.

The hormones were administered in similar total volumes of sesame oil while the two control birds received similar amounts of oil only.

EXPERIMENTAL RESULTS

The pullets remained in excellent condition throughout the experiment. Their initial and final live weights are presented in Table 1.

TABLE 1.—LIVE WEIGHTS OF EXPERIMENTAL PULLETS

Bird No.	Treatment	Live weight		
		Initial kgm.	Final kgm.	Increase kgm.
1	Nil	1.10	1.47	0.37
4	Nil	1.10	1.46	0.36
2	ODB	1.11	1.44	0.33
5	ODB	1.07	1.50	0.43
3	ODB + TST	1.08	1.58	0.50
6	ODB + TST	1.10	1.52	0.42

The results for daily Ca retention during the experimental period are plotted in Figure 1. Linear regressions of Ca retention on days for (a)

* Pulverizing Machinery Co., Summit, N.J.
† "Progynon-B", Schering, Ltd., Montreal.
‡ "Oreton", Schering, Ltd., Montreal.

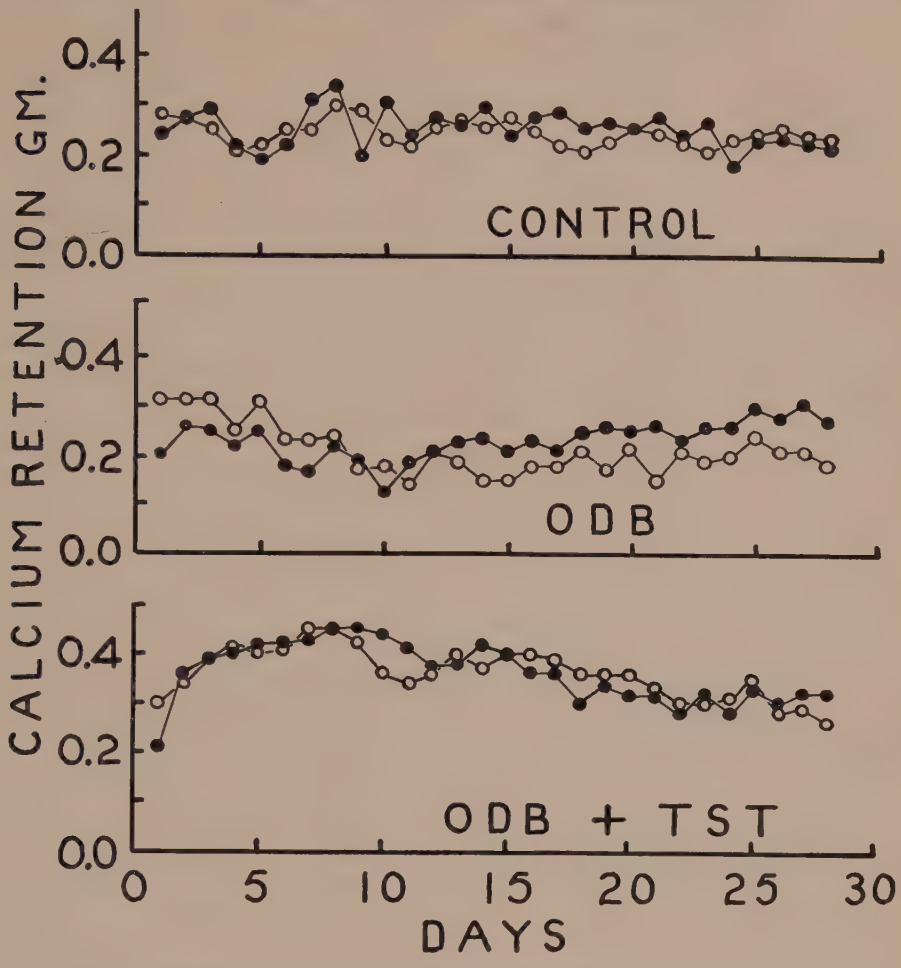

FIGURE 1. Effects of administration of estrogen and estrogen + androgen on calcium retention by immature pullets. Two pullets on each treatment.
The first dose of hormone was given at zero time, i.e., at the beginning of Day 1, hence the first values plotted relate to pullets coming under influence of exogenous hormones.

the first 10 days [8 days in the case of the birds receiving ODB + TST] and for (b) the second 18 days are presented in Table 2 and are shown graphically in Figure 2.

The retentions for the two control pullets over the first 10 days were somewhat variable, and the slight positive regression did not attain significance. However, the plots for the two pullets displayed a rather close correspondence in detail (Figure 1); the daily retention fell from about 0.27 gm. to about 0.20 gm. and then recovered to an average around 0.27 gm. by the tenth day. This initial decline and recovery may have been connected with exceptionally hot weather that coincided with the first week of the balance period. From Day 11 to Day 28 the Ca retention displayed a tendency to decline slowly, this tendency being highly significant.

TABLE 2.—LINEAR REGRESSIONS OF Ca RETENTION (IN GM.) ON TIME (IN DAYS) FOR SEXUALLY IMMATURE PULLETS TREATED WITH GONADAL HORMONES

Pullets	Treatment	Days 1–10 inclusive		Day 11–28	
		Regression	t	Regression	t
1 & 4	nil	$Y = +0.00223x + 0.242$	0.47	$Y = -0.00179x + 0.284$	2.75[2]
2 & 5	ODB	$Y = -0.1263x + 0.299$	2.74[1]	$Y = +0.00383x + 0.147$	8.70[2]
3 & 6	ODB + TST	$Y = +0.2256x + 0.288$[3]	3.17[2]	$Y = -0.00641x + 0.467$	4.34[2]

[1] Significant at P = 0.05
[2] Significant at P = 0.01
[3] Calculated for days 1–8 only (*see text*).

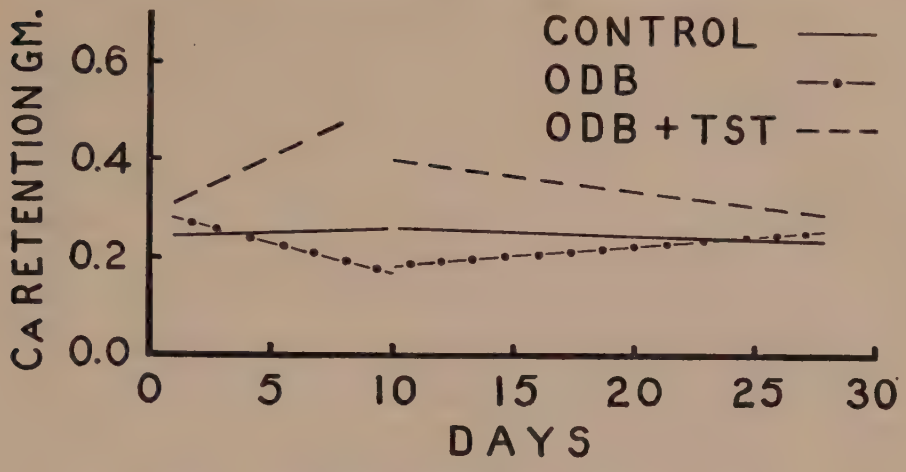

FIGURE 2. Mean linear regressions of Ca retention on time.

The daily retention by the pullets undergoing treatment with ODB + TST rose sharply from an average of 0.25 gm. for Day 1 to an average of 0.40 gm. for Day 4, and then more slowly to 0.45 gm. for Day 8. Thereafter the Ca retention declined rather steadily to levels around 0.30 gm. for Days 23 to 28. The regression for No. 3 and No. 6 is given for the first 8 days only because the obviously curvilinear relations lead to an unrealistic reduction of significance when the first 10 days are included. The regression for the decline in retention from Day 10 to Day 28 was highly significant. It may be remarked that the curve for the two ODB + TST birds up to Day 11 showed close agreement with that obtained previously by Jowsey *et al.* (5) for pullets undergoing a similar treatment. There were some slight indications of a dip in the curve for Days 4 and 8; this coincided with the dip in the curve for the control birds that has been mentioned above, and it may also have been a consequence of the spell of exceptionally hot weather.

The principal interest of the present experiment lies in the results for the birds that received ODB only. The plots for Ca retention (Figure 1) by these two birds displayed similar trends even though the two curves did not lie as close together as for the two other pairs. The Ca retention of both pullets fell rather regularly from their initial values to reach minimal values at Days 10 to 11. The negative regression of the combined results for the first 10 days was significant, as may be seen from Table 2. ODB alone did not elicit any marked transient increase of Ca retention, but from Day 5 onward the results from this treatment agreed with those previously observed (5). The absence of a transient increase of Ca retention may possibly have been due to the coincidence of the extremely hot weather mentioned above. From Day 11 to 28, the Ca retention of pullet No. 2 recovered steadily to a value higher than that observed during the first 5 days. Pullet No. 5 displayed a similar trend, though to a less pronounced degree. The positive regression for the last 18 days was highly significant. It is probable that the previous experiments of

TABLE 3.—AVERAGE SERUM Ca OF SEXUALLY IMMATURE PULLETS TREATED WITH GONADAL HORMONES

Hormonal treatment	Serum Ca mgm./100 ml.		
	Day 15	Day 26	Day 30[1]
Nil	10.9	11.3	11.0
ODB	38.0	47.0	46.2
ODB + TST	44.5	53.3	54.4

[1] The pullets were maintained under experimental conditions until this day although collections of droppings had ended.

Jowsey *et al.* (5) were terminated just when the average retention by their birds treated with ODB only was attaining minimal values. The present experiment establishes that the decline observed in the previous work is not a prolonged effect. Nevertheless, Ca retention by the birds receiving ODB by itself remained below that of the control birds over most of the second week, of the third and, to a lesser degree, of the fourth week of the treatment. It seems reasonable to infer that, in certain circumstances of dosage and nutritional status, estrogen alone will depress Ca retention by the immature pullet, at least for a time. The results for treatment with ODB + TST suggest that the negative effects of ODB on Ca retention could be offset by concurrent treatment with relatively small amounts of androgen.

It was thought possible that the serum Ca of the estrogenized birds might fall off with time. Mandel, Clavert and Mandel (7) have reported that the acid-soluble P of the serum of estrogenized ducks reverted to near the initial values after a month, despite continued treatment. These workers attributed this to an acquired refractoriness of the birds to estrogen. The limited number of serum Ca determinations made in the course of the present experiment did not reveal any analogous trends of the serum Ca values of the estrogenized pullets. In fact, serum Ca was greater at Day 30 than at Day 15. The relevant results are presented in Table 3. The results also accord with the observation that the hypercalcemia evoked by ODB is enhanced by concurrent administrations of TST, an observation that has been made repeatedly in this laboratory.

DISCUSSION OF RESULTS

Previous work has left little doubt that ODB by itself may give a transient increase in Ca retention by immature pullets. The present results suggest that this is not invariably observed; the succeeding decline in Ca retention by such birds is probably a more consistently reproducible phenomenon. The transient increase in Ca retention may be a reflection of slight endogenous androgen activity in the sexually immature pullet. If such activity were present, it would be rapidly suppressed by the exogenous estrogen, and this would account for the observed sequence of effects of ODB alone. Absence of a definite transient increase would, on this hypothesis, be merely a reflection of a particularly low level of endogenous androgen.

The decline of Ca retention from about Day 10 onward by the birds receiving estrogen plus androgen might be due to the onset of refractoriness to the hormonal treatment; or it may be that physiological equilibria were displaced rather rapidly toward a higher potential of mineralization and then held at this higher level; on this view the subsequent slower decline would then be regarded as due to the same factors as the much slower decline shown by the controls over the same period. The beginning of the decline preceded by only 2 days the time at which retentions by the ODB birds passed their minimal values; this suggests that there were readjustments of equilibria to the hormonal treatments and that the readjustments took roughly the same time for both groups. The slight decline in retention of the controls may have been merely an expression of the slowing-up of growth rate with advancing age. It is obvious that both shorter and longer term experiments are necessary to provide a complete picture of the relations between the action of gonadal hormones and calcium metabolism.

ACKNOWLEDGEMENTS

The authors wish to express their indebtdness to the National Research Council of Canada for financial support that defrayed part of the cost of equipment and materials, and to Messrs. Schering Ltd., Montreal, for generous gifts of hormonal preparations. They wish also to thank D. S. Layne for the serum calcium determinations, and L. A. Wright for his ready help in the laboratory.

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THE INHERITANCE OF RUST RESISTANCE.

I. THE INHERITANCE OF STEM RUST RESISTANCE IN TEN VARIETIES OF COMMON WHEAT[1]

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[Received for publication December 29, 1955]

ABSTRACT

The inheritance of resistance to races 15B and 56 of stem rust was studied in the varieties Kenya 58, Kenya 117A, Red Egyptian, Egypt Na95, McMurachy, Gabo, Lee, Timstein, Thatcher and Marquis. Rust tests were made on F_2 populations from diallel crosses and on families from backcrosses to the susceptible variety Marquis. The following genes are present in the varieties:

1. A gene governing a hypersensitive reaction to both races in Kenya 58, Red Egyptian and McMurachy.
2. A gene governing resistance to race 15B in Kenya 58, Kenya 117A and Egypt Na95.
3. A gene governing moderate resistance to both races in Red Egyptian.
4. A gene governing moderate resistance to race 56 in Red Egyptian, Kenya 117A and Egypt Na95.
5. A gene governing resistance to race 56 in Kenya 117A and Egypt Na95.
6. Two complementary genes governing resistance to race 56 in Gabo, Lee and Timstein.

INTRODUCTION

The rapid increase in North America of race 15B of wheat stem rust, *Puccinia graminis tritici* Eriks. and Henn., has emphasized the need for clarification of the inheritance of rust resistance in common wheat, *Triticum vulgare* Vill[3]. Since 1950 race 15B has caused considerable damage to the wheat crops in Canada and the United States. Until the release of Selkirk in 1953 by the Cereal Breeding Laboratory at Winnipeg, no variety of hard red spring wheat, resistant to race 15B, was available.

Extensive research on rust resistance in wheat has been carried on for many years and has been notably successful in providing farmers with rust resistant varieties. In the development of these varieties, studies have been carried out on many of the segregating populations from crosses and considerable genetic data are available. Most of these data have not been integrated. Little is known about either the number of loci involved in resistance or the genes carried by various resistant varieties.

The work reported in this paper is part of a program to determine more about the inheritance of rust resistance in common wheat. For the present, two races of stem rust are being used, races 15B and 56. In this first paper the inheritance and interrelationships of the genes for resistance to these races are reported for the varieties Kenya 58, Kenya 117A, Red Egyptian, Egypt Na95, McMurachy, Gabo, Lee, Timstein, Thatcher and Marquis.

[1] Work reported in this paper was done under a grant from the Canada Department of Agriculture for Extra-mural Research Project EMR-16. The second author was supported in part by a studentship from the National Research Council. A portion of the work was submitted by the second author to the University of Saskatchewan in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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[3] It is realized that *Triticum aestivum* L. has priority; however, *Triticum vulgare* Vill. is retained because of its common usage in the literature.

REVIEW OF LITERATURE

Ausemus (2) reviewed the literature on rust resistance in wheat, while Ausemus *et al.* (3) summarized the work on the inheritance of rust resistance and suggested symbols for the known genes. Only those papers dealing with the varieties used in this study will be reviewed here. Brief descriptions of the origin and rust reaction of the varieties are included in this section since they are helpful in interpreting the literature reports.

Kenya 58, Red Egyptian, Kenya 117A, Egypt Na95 and McMurachy all have good resistance to races 15B and 56 at moderate temperature, but their resistance breaks down at high temperatures.

Kenya 58 was reported by Johnson (7) to be a cross between Red Egyptian and a Kabete hybrid (named for the place where it was developed) and may have obtained rust resistance from both parents. Hasanain (5) studied a cross between Pusa 4 and Kenya 58 and concluded that in Kenya 58 resistance to race 15B is controlled by three factors which are equal and cumulative in effect.

Red Egyptian and McMurachy have been shown by Peterson and co-workers (see Goulden, 4) and by Shebeski (16) to have in common a single recessive gene which governs resistance to race 15B and to other common races. The parentage of Red Egyptian is unknown, but apparently it came from Ethiopia. McMurachy was discovered as a single rust-free plant in a field of Garnet wheat in Manitoba and was named after the farmer who found it. Peterson and Masson (11) found that McMurachy carried a single recessive gene for rust resistance. Peterson and Campbell* used monosomics to analyse McMurachy and found it to have a single partially dominant gene on chromosome XX. As reported by Sears (14), Sears and Rodenhiser located one gene in Red Egyptian on chromosome XX and one on chromosome VI. Each gene governed resistance to a number of races including 15B and 56. A third gene which provides some resistance to race 17 and 56 has been located on chromosome XIII by Sears* and Loegering.

Kenya 117A was reported by Johnson (7) to have come from a cross between a Njoro hybrid and Marquis. Athwal and Watson (1) showed that two genes for rust resistance are present in Kenya 117A. One gene governs resistance to the Australian races and to race 38, while the second governs resistance to races 38 and 122.

Egypt Na95 was introduced from Kenya and resulted from a cross between Kenya U and 9 M.I.A.3. Macindoe (9) and Shebeski (16) both worked with Egypt Na95 and had difficulty analysing their results. Shebeski found Egypt Na95 to be heterogeneous. Macindoe suggested that one gene was responsible for resistance to the races which he used.

Gabo, Lee and Timstein are very similar in their rust reaction. All three are moderately resistant to race 56 but have only slight resistance to race 15B.

Gabo was reported by Watson and Waterhouse (20) to have come from the cross (Bobin × Gaza durum) × Bobin and it obtained its rust resistance from Gaza. Macindoe (9) found that the resistance of Gabo

* *Personal communications.*

to race 34 is conditioned by a single dominant factor. Watson (19) reported that Gabo has three independent factors for resistance to the Australian races of rust.

Timstein was produced in Australia by Pridham (13) from a cross between *Triticum timopheevi* Zhukov and a common wheat, Steinwedel, and obtained its rust resistance from the *T. timopheevi* parent. Sears and Rodenhiser (15) showed that Timstein has two dominant, complementary genes for resistance to many races including race 56. By means of nullisomic analysis they located both genes on chromosome X. In crosses not involving nullisomic X, the ratio of resistant to susceptible plants deviated sufficiently from 9 : 7 to indicate linkage. Koo and Ausemus (8) studied crosses of Timstein with Thatcher, Newthatch and Mida and concluded that Timstein carries a single factor which governs seedling resistance to 20 races, including race 56.

Lee was developed in Minnesota from the cross Hope × Timstein. Plessers (12) used monosomics to analyse Lee and showed that seedling resistance to a number of races, including race 56, is conditioned by two dominant, complementary genes on chromosome X. He obtained a recombination value of 29.5 per cent.

Thatcher has moderate resistance to race 56 but is susceptible to race 15B. As reported by Hayes *et al.* (6), Thatcher was developed in Minnesota from the cross (Marquis × Iumillo) × (Kanred × Marquis) and probably obtained rust resistance from both Iumillo and Kanred. Different authors have reported that Thatcher has either two or three recessive factors for rust resistance. Swenson *et al.* (18) suggested that at least two or three recessive genes were involved. Koo and Ausemus (8) presented evidence that field resistance to a mixture of races was determined by two recessive genes. Sears and Rodenhiser as reported by Sears (14) located one gene for seedling resistance on chromosome XIX.

Marquis is very susceptible to both race 15B and race 56. It was developed at Ottawa from the cross Hard Red Calcutta × Red Fife.

The naming of the genes in wheat has recently been the subject of considerable discussion. Ausemus *et al.* (3) suggested that genes for rust resistance be labelled with the symbol *Sr* plus an Arabic number. Where the relationship of a new gene to those previously identified is not known, they recommended that a subscript letter, preferably the first letter of the variety involved, be used temporarily. Recently, Athwal and Watson (1) have developed an extension of this system to name the genes in certain Kenya varieties. They used a subscript which included the letter *K* for Kenya, a lower case letter to indicate the variety and a number to indicate the particular locus in that variety (e.g. Sr_{Kal}). In addition a superscript lower case letter was used to distinguish alleles where sufficient evidence was not available to prove them identical. It is evident that this system is somewhat complicated. In the present paper the first system, using the symbol *Sr* plus an Arabic number, has been employed. Temporary designations have not been used since it is unlikely that any genes reported here are alleles of the genes named by Ausemus *et al.*

MATERIAL AND METHODS

Seed of the varieties Kenya 58 (C.I. 12471), Egypt Na95 (C.I. 12894), Red Egyptian (C.I. 12345), Gabo (C.I. 12795), Timstein (C.I. 12347), Lee (C.I. 12488) and Marquis (C.I. 3641) was obtained from the University of Minnesota. Kenya 117A (C.I. 13140) and McMurachy (C.I. 11876) came from the Laboratory of Cereal Breeding at Winnipeg while Thatcher (C.I. 1003) came from the University of Saskatchewan.

Diallel crosses were made between the nine varieties Kenya 58, Kenya 117A, Egypt Na95, Red Egyptian, Gabo, Timstein, Lee, Thatcher and Marquis. McMurachy was crossed with Kenya 58, Kenya 117A, Egypt Na95 and Red Egyptian. All of the varieties except McMurachy were backcrossed to Thatcher and all except McMurachy, Gabo and Timstein were backcrossed to Marquis. The crosses of Gabo and Timstein with Marquis produced only sterile dwarfs and no backcrosses were possible. The backcrosses to Thatcher were made primarily for plant breeding purposes while the backcrosses to Marquis were intended largely for genetic studies.

Races 15B and 56 of stem rust were used in the studies. Initial inoculum of both races was provided by the Plant Pathology Laboratory at Winnipeg and was then increased and maintained at Saskatoon. The original inoculum of race 15B contained a mixture of isolates found on the Canadian prairies, but did not include 15B-3. However, in none of the tests were there found mixed reactions on individual plants and it was concluded that resistance to all these isolates is controlled by the same genes.

Rust tests were conducted on the various generations of the crosses and backcrosses as noted below. The parents were included in all tests.

1. A few F_1 plants from each cross were tested for seedling reaction to races 15B and 56 and for field reaction to race 56.
2. Large F_2 populations (up to 1500 plants) from each cross were tested for field reaction to race 56. Additional F_2 plants from the crosses between Kenya 58, Kenya 117A, Egypt Na95, Red Egyptian and Marquis were tested for seedling resistance to both races.
3. From each backcross to Marquis up to 100 F_2 families were tested for seedling resistance to both races. Backcross families, representing each of the possible genotypes for seedling rust resistance, were tested for field reaction to race 15B to determine whether the seedling resistance carried through to maturity and whether additional factors for mature plant resistance were present.

Field rust tests were conducted in an irrigated nursery. The material under study was planted in paired rows 6 inches apart with 18 inches between pairs. After every fifth pair a single row was planted to a mixture of susceptible varieties. As soon as the stems on the plants in these "spreader" rows were elongating, one stem about every two feet was inoculated with a suspension of spores of a single race by means of a hypodermic needle. In 1953 race 56 was used and, although natural inoculum of race 15B arrived late in the summer, only race 56 was identified from the nursery. In 1954 race 15B was used and was supplemented by a very early and heavy natural infection of race 15B.

Plants from field tests were pulled and the percentage rust was read for each plant using the scale outlined by Peterson *et al.* (10).

For seedling tests in the greenhouse, plants were grown in beds 20 feet by 3.5 feet. Rows were spaced 3 inches apart and 50–60 seeds planted per row. The seedlings were inoculated in the two leaf stage using the following method:

1. The leaves were wetted by means of a very fine weed spray nozzle connected to a hose and were then rubbed between the fingers to remove the bloom.
2. The leaves were wetted again and then dusted with a mixture of approximately 1 part rust spores to 5 parts of talc.
3. A heavy canvas, shaped like the top of a box, was fitted over the entire bed and left for 24 hours. During this time the canvas and the seedlings were sprayed periodically.

In general very uniform rust infections were obtained.

The seedling tests were run in the spring and fall when greenhouse temperatures could be kept reasonably low. No indication of a high temperature breakdown of resistance was found in any test.

With some material, in order to save time, space and seed, a method of testing the same plants with two races of rust was used. At the early two-leaf stage the plants were inoculated with the first race and 7 to 8 days later with the second race. After two weeks the first infection was read and the rusted leaves removed before the second infection developed. After three weeks the second infection was read and the plants discarded. No evidence was found to indicate that the second infection interfered in any way with the first. Parent varieties gave their typical reactions to both races. For several backcrosses each of two lots of seedlings was tested with a single race and a third lot of seedlings was tested with both races. The results for each race were the same in both types of test.

Rusted seedlings were read according to pustule type using the system set up by Stakman *et al.* (17).

The Chi-Square test for goodness of fit was used in analysing the results. The correction necessary for enumeration data was applied in all cases.

TABLE 1.—SUMMARY OF RUST TESTS ON PARENTS AND F_2 POPULATIONS FROM THE DIALLEL CROSSES.

	Variety and rust reaction	Race 56—seedling and field tests								
		Mar. S	K. 58 VR	R.E. VR	K.117A R	Na 95 R	Gabo MR	Lee MR	Tim. MR	That. MR
Race 15B—Seedling tests	Marquis (S)		Seg.	Seg.	Seg.	Seg.	D	Seg.	D	Seg.
	Kenya 58 (VR)	Seg.		VR	Seg.	Seg.	Seg.	Seg.	Seg.	Seg.
	Red Egyptian (VR)	Seg.	VR		Seg.	Seg.	D	Seg.	D	Seg.
	Kenya 117A (R)	Seg.	VR–R	Seg.		R	Seg.	Seg.	Seg.	Seg.
	Egypt Na95 (R)	Seg.	VR–R	Seg.	R		Seg.	Seg.	Seg.	Seg.
	Gabo (MS-S)							MR	MR	Seg.
	Lee (MS-S)								MR	Seg.
	Timstein (MS-S)									Seg.
	Thatcher (S)									

VR = very resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, Seg. = segregating, D = dwarf F_1.

RESULTS

Throughout this paper the results of tests on F_2 populations are used primarily to show whether or not two varieties have a gene or genes in common. The genetic analysis of each variety is based largely on the data from backcrosses to the rust susceptible parent Marquis. Dominance of the various genes is determined from the ratios obtained in backcross families in which a single gene for resistance was segregating.

A complete summary of F_2 data is given in Table 1. For the sake of simplicity, the data will not be considered as a whole but will be discussed separately under each variety.

Kenya 58

The data from rust tests on the cross and backcross of Kenya 58 to Marquis are given in Tables 2 and 3. The number of backcross families is small and, for this reason, data from a few backcrosses to Thatcher are included. Fortunately, the segregations within families were clearcut and this tends to compensate for the lack in number of families.

When tested with race 56, the F_2 families from the backcross to Marquis gave a satisfactory fit to a ratio of 1 segregating: 1 susceptible.

TABLE 2.—RESULTS OF SEEDLING TESTS ON F_2 FAMILIES FROM THE BACKCROSS OF KENYA 58 TO MARQUIS AND THATCHER.

	Race 15B				Totals (race 56)	Expected (1 : 1)	P
	Number of families						
	Seg. 1VR : 3S	Seg. 4VR:9MR:3S	Seg. 3MR : 1S	S			
Kenya 58 × Marquis²F₂							
Race 56 Seg. 3VR : 1S	10	6			16[1]	13	.30–.50
Susc.			3	7	10	13	
Totals (race 15B)	10[2]	6[3]	3[4]	7	26	26	
Expected (1 : 1 : 1 : 1)	6.5	6.5	6.5	6.5	26		
P	.30–.50						
Kenya 58 × Thatcher²F₂ Number of families	5[5]	11[6]	6[7]	11	33		
Expected (1 : 1 : 1 : 1)	8.25	8.25	8.25	8.25	33		
P	.30–.50						

The ratios within segregating families were as follows:

Race 56

[1] 510 VR : 165S plants — P for a 3 : 1 ratio = .70–.80

Race 15B

[2] 103 VR : 338S plants — P for a 1 : 3 ratio = .30–.50
[3] 74 VR : 141MR : 59S plants — P for a 4 : 9 : 3 ratio = .30–.50
[4] 136MR : 32S plants — P for a 3 : 1 ratio = .05–.10
[5] 50 VR : 155S plants — P for a 1 : 3 ratio = .90
[6] 124 VR : 252MR : 99S plants — P for a 4 : 9 : 3 ratio = .30–.50
[7] 204MR : 89S plants — P for a 3 : 1 ratio = .02–.05

(the poor fit is due to one family which segregated 26 : 17)

Within the segregating families a ratio of 3 very resistant seedlings (fleck to type 1^-): 1 susceptible (type 4) was obtained. The results indicate that a single dominant gene, which will be called *Sr6**, controls seedling resistance to race 56.

Of the sixteen families from the backcross to Marquis which segregated for the fleck reaction to race 56, all 16 also segregated for a fleck reaction to race 15B. Apparently, *Sr6* controls resistance to both races (further evidence on this point is given at the end of the section). However, while *Sr6* behaved as a dominant gene for seedling resistance to race 56, it behaved as a recessive for resistance to race 15B. Thus, in the tests with race 15B the seedlings in the 16 families segregated in a ratio of 1 very resistant (fleck to type 1^-): 3 of other types.

A second type of resistance to race 15B segregated in 9 of the backcross families and a second independent gene, *Sr7*, is postulated. The families segregating for *Sr7* alone gave a ratio of 3 moderately resistant seedlings: 1 susceptible. In families segregating for both *Sr6* and *Sr7* a good fit was obtained to a ratio of 4 very resistant: 9 moderately resistant : 3 susceptible seedlings. The dominance of *Sr7* was, however, not complete. Heterozygous plants frequently carried type 3 pustules compared to type 1 to 1^+ for homozygotes. The heterozygotes were, however, readily separated from susceptible plants on the basis of a typical chlorosis around their pustules, particularly at the tips of the leaves. The gene *Sr7* apparently has no effect on resistance to race 56.

The results of tests with race 15B on the Thatcher backcrosses substantiate the above hypothesis.

When representative backcross families were tested with race 15B in the field no evidence for genes conditioning mature plant resistance alone was found. As was the case in the seedlings, *Sr6* provided better resistance than did *Sr7*.

* Ausemus *et al.* (3) have recommended the use of the symbols Sr_1–Sr_5 for previously identified genes.

TABLE 3.—RESULTS FROM RUST TESTS ON F_2 POPULATIONS FROM THE CROSS KENYA 58 × MARQUIS

Variety or cross and type of test		Number of plants			Ratio	P
		VR	MR	S		
Kenya 58						
—race 56		All				
—race 15B		All				
Kenya 58 × Marquis F_2						
Seedling tests	Observed	20	29	13		
—race 15B	Expected	15.5	34.9	11.6	4:9:3	.30–.50
Seedling tests	Observed	48		22		
—race 56	Expected	52.5		17.5	3:1	.20–.30
Field tests	Observed	276	512	288		
—race 56	Expected	269	538	269	1:2:1	.20–.30

TABLE 4.—RESULTS OF SEEDLING TESTS ON F_2 FAMILIES FROM THE BACKCROSS TO MARQUIS OF PLANTS HOMOZYGOUS FOR Sr_6

		Race 15B		Totals (race 56)	Expected (1:1)	P
		Number of families				
		Seg.1VR:3S	S			
Race 56	Seg. 3VR : 1S	122		122[1]	115.5	.30–.50
	S		109	109	115.5	
Totals (race 15B)		122[2]	109	231	231	
Expected (1 : 1)		115.5	115.5	231		
P		0.30–0.50				

The ratios within segregating families were as follows:

Race 56

[1] 1744VR : 583S plants — P for a 3 : 1 ratio = .90–.95

Race 15B

[2] 569VR : 1766S plants — P for a 1 : 3 ratio = .30–.50

The data from the tests on F_2 plants (Table 3) corroborate the backcross results. In the seedling tests with race 15B a good fit to a ratio of 4 very resistant : 9 moderately resistant : 1 susceptible was obtained. When the same plants were tested with race 56 a good fit for a ratio of 3 very resistant: 1 susceptible was obtained. All 20 plants which were very resistant to race 15B were also very resistant to race 56. In the field tests with race 56, three distinct classes of plants were present—very resistant (less than 1 per cent rust), moderately resistant (5–50 per cent rust) and susceptible (80 per cent or more rust). The numbers of plants in the three classes gave a good fit to a 1 : 2 : 1 ratio. Apparently, the resistance to race 56 provided by *Sr6* is almost completely dominant in seedlings but only incompletely dominant in mature plants.

The *Sr6* gene is an unusual one. Its behaviour as a dominant for resistance to race 56 and as a recessive for resistance to race 15B suggested the possibility that not one but two closely linked genes were involved. To test this, plants that were homozygous for *Sr6* and lacked *Sr7* were crossed and backcrossed to Marquis. Plants from F_2 backcross families were then inoculated twice, first with race 15B and then with race 56. The results are given in Table 4. The expected ratios, 1 very resistant : 3 susceptible plants with race 15B and 3 very resistant : 1 susceptible with race 56, were obtained. None of the 231 families segregated for resistance to only one race. Within the 122 segregating families all 569 plants which were resistant to race 15B were also resistant to race 56. If more than one gene is involved then the linkage must be close. It was noted in the tests with race 56 that plants which were homozygous for *Sr6*

TABLE 5.—RESULTS OF SEEDLING TESTS ON F_2 FAMILIES FROM THE BACKCROSS OF RED EGYPTIAN TO MARQUIS

		Race 15B — Number of families				Totals (race 56)	Expected (1 : 1 : 2 : 1 : 1 : 1 : 1)	P
		Seg. 1VR : 3S	Seg. 4VR : 12MR-S	Seg. MR-S	Susc.			
Race 56	Seg. 3VR : 1S	9				9[1]	12	
	Seg. 12VR : 3MR : 1S	10				10[2]	12	
	Seg. 12VR : 3MR : 1S or 48VR : 15MR : 1S		35			35[3]	24	.20–.30
	Seg. 3MR : 1S			10		10[4]	12	
	Seg. 15MR : 1S			13		13[5]	12	
	Seg. 3MR : 1S				12	12[6]	12	
	Susc.				7	7	12	
Totals (race 15B)		19[7]	35[8]	23[9]	19	96	96	
Expected (1 : 1 : 1 : 1)		24	24	24	24	96		
P		0.05–0.10						

The ratios within segregating families were as follows:

Race 56

[1] 155 VR : 59S plants — P for a 3 : 1 ratio = .30–.50
[2] 188 VR : 42MR : 18S plants — P for a 12 : 3 : 1 ratio = .70–.80
[3] 713 VR : 192MR : 34S plants — P for a 48 : 13.5 : 2.5 ratio = .80–.90 (combination of 12 : 3 : 1 and 48 : 15 1)
[4] 196MR : 57S plants — P for a 3 : 1 ratio = .30–.50
[5] 303MR : 13S plants — P for a 15 : 1 ratio = .10–.20
[6] 255MR : 65S plants — P for a 3 : 1 ratio = .05–.10

Race 15B

[7] 153 VR : 419S plants — P for a 1 : 3 ratio = .30–.50
[8] 257 VR : 758MR-S plants — P for a 4 : 12 ratio = .80–.90
(The 758 plants in the second group could not be separated into distinct classes)
[9] The plants in these families could not be separated into distinct classes

TABLE 6.—RESULTS FROM RUST TESTS ON F_2 POPULATIONS FROM THE CROSSES RED EGYPTIAN × MARQUIS AND KENYA 58 × RED EGYPTIAN

Variety or cross and type of test		Number of plants VR	MR	S	Ratio	P
Red Egyptian						
Race 15B		All				
Race 56		All				
Red Egyptian × Marquis F_2						
Seedling tests —race 15B	Observed	20	34	16		
	Expected	17.5	39.4	13.1	4 : 9 : 3	.50–.70
Seedling tests —race 56	Observed	91	29	1		
	Expected	90.7	28.4	1.9	48 : 15 : 1	.95–.98
Kenya 58 × Red Egyptian F_2						
Field tests —race 56		1039		0		
Seedling tests —race 15B		144		0		
Seedling tests —race 56		135		0		

(and consequently resistant to race 15B) showed very tiny flecks while the heterozygotes had either larger flecks or extremely small type 1 pustules.

Red Egyptian

The critical data from rust tests on crosses and backcrosses involving Red Egyptian are given in Tables 5 and 6.

Three facts are clear in the data from the backcrosses to Marquis (Table 5). First, it is evident that 54 (9 + 10 + 35), or about half of the backcross families, segregated for a very resistant reaction (fleck to type 1^-) to both race 15B and race 56. Similarly, 58 (35 + 10 + 13) families segregated for a moderately resistant reaction (type 2–2^+) to both races. Lastly, of the 38 families which did not segregate for moderate resistance to race 15B, 22 or about half did segregate for a moderately resistant (type 2–2^+) reaction to race 56. These last 38 families comprise the 19 that were susceptible to race 15B plus the 19 that segregated for only the very resistant reaction to race 15B. The data fit the hypothesis that one gene in Red Egyptian conditions a hypersensitive type of resistance to both races, a second gene conditions moderate resistance to both races and a third gene conditions moderate resistance to race 56 but not to race 15B. As is shown below, the validity of the hypothesis is substantiated by the segregations within families.

The gene which conditions a hypersensitive type of resistance is identical in its action to gene *Sr6* of Kenya 58. In tests with race 15B the seedlings of the 54 families segregating for this gene gave a ratio of 1 very resistant : 3 of other types. With race 56 all 54 families gave ratios of 3 very resistant seedlings : 1 of other types. It was not surprising, then, that all F_2 seedlings from the cross Kenya 58 × Red Egyptian gave a fleck reaction to both races and F_2 plants were highly resistant to race 56 in the field (Table 6). The results indicate that both varieties carry *Sr6*.

The second Red Egyptian gene is not similar to gene *Sr7* of Kenya 58. It differs in the type of resistance it conditions to race 15B and in addition provides resistance to race 56 which *Sr7* does not. This gene will, therefore, be called *Sr8*. Seedlings homozygous for *Sr8* exhibited a type 2 to 2^+ reaction to both races while heterozygotes ranged from 2^+ to 3^+ or even 4^-. In general seedlings were somewhat more resistant to race 56 than to race 15B. For this reason, within the families segregating for *Sr8* no clear ratios were obtained in the tests with race 15B but satisfactory ratios were obtained with race 56. When tested with race 56 the plants in the ten families segregating only for *Sr8* gave a good fit to a ratio of 3 moderately resistant : 1 susceptible (Table 5, footnote 4). In families segregating for both *Sr6* and *Sr8* the segregations for resistance to race 56 gave a good fit to either a ratio of 12 very resistant : 3 moderately resistant : 1 susceptible or a ratio of 48 very resistant : 15 moderately resistant : 1 susceptible, depending on whether or not the third gene was present (Table 5, footnote 3). In the field *Sr8* provides only poor resistance to race 15B.

The third gene in Red Egyptian will be called *Sr9*. It is an incompletely dominant gene which conditions a 2 to 2^+ reaction to race 56. In the tests with race 56 heterozygous plants were less resistant than homozygotes but could usually be separated from fully susceptible plants. In the 12

TABLE 7.—RESULTS OF SEEDLING TESTS ON F_2 FAMILIES FROM THE BACKCROSS OF KENYA 117A TO MARQUIS

		Race 15B Number of families		Totals (race 56)	Expected (3 : 1)	P
		Seg.	S			
Race 56	Seg.	40	36	76	81.75	.20–.30
	S	17	16	33	27.25	
Totals (race 15B)		57[1]	52	109	109	
Expected (1 : 1)		54.5	54.5	109		
P		.70–.80				

The ratio within families segregating for resistance to race 15B was as follows: P for a 3 : 1 ratio = .30–.50

[1] 1458MR : 506S plants

TABLE 8.—RESULTS OF RUST TESTS ON F_2 POPULATIONS FROM CROSSES OF KENYA 117A WITH MARQUIS, KENYA 58 AND RED EGYPTIAN

Variety or cross and type of test		Number of plants VR	R or MR	S	Ratio	P
Kenya 117A						
Seedling tests —race 15B			All			
Seedling tests —race 56			All			
Kenya 117A × *Marquis* F_2						
Seedling tests —race 15B	Observed	0	83	26		
	Expected	0	81.8	27.2	3 : 1	.80–.90
Seedling tests —race 56	Observed	0	79	8[1]		
	Expected	0	81.6	5.4	15 : 1	.30–.50
Kenya 117A × *Kenya 58* F_2						
Seedling tests —race 15B	Observed	159	58	0		
	Expected	162.8	54.2	0	3 : 1	.50–.70
Seedling tests —race 56	Observed	156	51	6		
	Expected	159.8	49.9	3.3	48 : 15 : 1	.30–.50 C
Field tests —race 56	Segregated—0–80% rust (390 plants)					
Kenya 117A × *Red Egyptian* F_2						
Seedling tests —race 15B	Observed	38		1		
	Expected	38.4		.6	63 : 1	1.0
Seedling tests —race 56	Observed	42		0		
	Expected	—		—		
Field tests —race 56	0–40% rust (1320 plants)					

[1] One aberrant family segregated 15MR : 5S and is omitted from these figures.

families in which only *Sr9* was segregating, the seedlings gave an acceptable fit to a ratio of 3 moderately resistant : 1 susceptible (Table 5, footnote 6). In the 13 families which were segregating for both *Sr8* and *Sr9* an acceptable fit to a ratio of 15 moderately resistant seedlings : 1 susceptible to race 56 was obtained (Table 5, footnote 5). Further, in the families segregating for both Sr_6 and Sr_9 a good fit to a ratio of 12 very resistant seedlings : 3 moderately resistant : 1 susceptible to race 56 was obtained (Table 5, footnote 2).

The segregations in the F_2 population from the cross Red Egyptian × Marquis also fit the expected ratios, 4 very resistant : 9 moderately resistant: 3 susceptible seedlings to race 15B and 48 very resistant : 15 moderately resistant : 1 susceptible to race 56.

Kenya 117A

The results of rust tests on plants from crosses and backcrosses involving Kenya 117A are given in Tables 7 and 8.

When tested with race 15B, the F_2 families from the backcrosses of Kenya 117A to Marquis gave a good fit to a ratio of 1 segregating : 1 susceptible. Within the segregating families a good fit to a ratio of 3 moderately resistant plants : 1 susceptible was obtained. A good fit to a 3 : 1 was also obtained in the F_2 of the cross Kenya 117A × Marquis. It is clear that Kenya 117A has a single gene for resistance to race 15B. The type of resistance conditioned by this gene was exactly like that conditioned by the *Sr7* gene of Kenya 58. The typical reaction of a homozygous seedling was type 1 or 1^+ while the heterozygotes ranged as high as type 3. All resistant plants showed the typical yellow chlorosis around the pustules. The F_2 seedlings from the cross Kenya 117A × Kenya 58 were all either very resistant or moderately resistant to race 15B. Evidently, gene *Sr7* is common to the two varieties.

An unexpected feature of the cross Kenya 117A × Kenya 58 was the number of plants having a more resistant reaction to race 15B than that typical of plants homozygous for the *Sr7* gene. Only the one-fourth of the seedlings which were homozygous for *Sr6* were expected to exhibit a fleck reaction while actually three-fourths did. The data would seem to indicate that in the presence of homozygous *Sr7*, a single *Sr6* gene is sufficient to produce the hypersensitive type of reaction whereas in previously mentioned crosses only plants homozygous for *Sr6* were resistant to race 15B. Actually, however, as will be shown in later sections, *Sr6* behaved as a dominant gene for resistance to race 15B in a number of crosses in which *Sr7* was not homozygous.

In the tests with race 56, the backcrosses to Marquis gave a satisfactory fit to a ratio of 3 segregating families : 1 susceptible. The plants within the segregating families could not be separated with certainty into classes. However, some segregations fitting a ratio of 3 moderately resistant plants : 1 susceptible and some fitting a ratio of 15 moderately resistant : 1 susceptible were noted. It is concluded that Kenya 117A has two partially dominant genes for resistance to race 56. In the F_2 population from the cross Kenya 117A × Marquis, segregation into classes for resistance to race 56 was more clear-cut and a good fit to a ratio of 15 moderately resistant plants : 1 susceptible was obtained. The two genes apparently

condition somewhat similar types of resistance to race 56. In the backcrosses to Marquis it was impossible to tell with certainty which families were segregating for one gene and which for the other.

The F_2 plants from the cross Kenya 117A × Kenya 58 segregated for resistance to race 56 in both the seedling and mature plant stages and it is apparent that the two varieties do not have a gene in common. In the seedling tests no difficulty was experienced in separating the moderately resistant from the susceptible plants and a good fit to the expected ratio of 48 very resistant plants : 15 moderately resistant : 1 susceptible was obtained.

The cross Kenya 117A × Red Egyptian segregated for resistance to race 15B indicating that the two varieties do not have a gene for resistance in common. Again in this cross *Sr6* behaved as a dominant gene for resistance to race 15B. Due to poor greenhouse conditions for rust

TABLE 9.—RESULTS OF SEEDLING TESTS ON F_2 FAMILIES FROM THE BACKCROSS OF EGYPT NA95 TO MARQUIS

Type A Plants

		Race 15B Number of families: Seg. 3MR : 1S	Race 15B Number of families: Susc.	Totals (race 56)	Expected (1 : 1)	P
Race 56	Seg. 3MR : 1S	4	17	21[1]	21	1.0
	Susc.	8	13	21	21	
	Totals (race 15B)	12[2]	30	42	42	
	Expected (1 : 1)	21	21	42		
	P	.01				

Type B Plants

		Race 15B Number of families: Seg. 3MR : 1S	Race 15B Number of families: Susc.	Totals (race 56)	Expected (2 : 1 : 1)	P
Race 56	Seg. 3MR : 1S	14	11	25[3]	25	.50–.70
	Seg. 15MR : 1S	5	5	10[4]	12.5	
	Susc.	6	9	15	12.5	
	Totals (race 15B)	25[5]	25	50	50	
	Expected (1 : 1)	25	25	50		
	P	1.0				

The ratios within segregating families were as follows:

Race 56

[1] 359MR : 115S plants — P for a 3 : 1 ratio = .70–.80
[3] 497MR : 165S plants — P for a 3 : 1 ratio = .95–.98
[4] 248MR : 23S plants — P for a 15 : 1 ratio = .10–.20

Race 15B

[2] 203MR : 68S plants — P for a 3 : 1 ratio = .95–.98
[5] 480MR : 193S plants — P for a 3 : 1 ratio = .02–.05

development, the separation of the resistant plants into classes was not clear-cut but about three-fourths did show a hypersensitive reaction. The segregation of resistant and susceptible plants fitted the 63 : 1 ratio expected on the assumption that three dominant genes for resistance to race 15B were segregating. However, the number of plants tested was too small for the segregation to have much significance. When tested with race 56 the cross Kenya 117A × Red Egyptian appeared not to segregate. Only 42 seedlings were tested and none was more susceptible than type 2. In the field 1320 plants were tested and only 2 were classified as high as 40 per cent rust and only 11 carried more than 10 per cent rust, although Marquis in the same nursery was read as 80 per cent. It seems probable, therefore, that Red Egyptian and Kenya 117A have in common a gene for resistance to race 56. The only gene in Red Egyptian which provides resistance to race 56 and not to race 15B is *Sr9* and it, then, would be the gene that is carried by both varieties. There is a possibility that the failure to get a susceptible plant in the F_2 population was not due to the presence of a common gene but to the segregation of 5 independent dominant genes, 2 from Kenya 117A and 3 from Red Egyptian. If this is the case, a 5 gene ratio would be expected with only 1 plant in 1024 being susceptible. The failure to obtain a susceptible plant in the large F_2 population tested in the field makes this unlikely but further crosses are being made to prove the hypothesis. It is evident that if the hypothesis is correct then *Sr9* provides only moderate resistance to race 56 in the field.

The second gene for resistance to race 56 in Kenya 117A is independent of the genes so far mentioned and is, therefore, named *Sr10*.

A few backcrosses to Marquis were tested in the field with race 15B. It was found that families which were known to be segregating for seedling resistance to both races 15B and 56 were more resistant than families which were segregating only for resistance to race 15B. Apparently one or both of the genes which by themselves have no effect on reaction to race 15B act as modifiers of the resistance conditioned by *Sr7*.

Egypt Na95

Egypt Na95 proved to be a difficult variety with which to work. Some of the plants used as parents were found to be heterozygous for rust resistance and a number of crosses had to be repeated. In addition several unexpected ratios have not been explained.

The results of rust tests on crosses and backcrosses involving Egypt Na95 are given in Tables 9 and 10.

Five F_1 plants from the cross Egypt Na95 × Marquis were backcrossed to Marquis. The progeny of two of these plants gave results that were difficult to analyse (Table 9, type A) while the progeny of the other three plants gave what were considered to be normal ratios (Table 9, type B). When tested with race 56, 21 or exactly half of the backcross families derived from the first two F_1 plants segregated for resistance. Within families a good fit to a ratio of 3 moderately resistant plants : 1 susceptible was obtained. It appeared, therefore, that a single dominant gene was segregating. In the tests with race 15B, 12 of the 42 families segregated. Such a result (a 1 : 3 ratio) would be obtained if resistance depended on two complementary genes. However, the segregations within families gave a

TABLE 10.—RESULTS OF RUST TESTS OF F_2 POPULATIONS FROM CROSSES INVOLVING EGYPT NA95

Variety or cross and type of test		Number of plants			Ratio	P
		VR	R or MR	S		
Egypt Na95						
Seedling tests —race 15B			All			
Seedling tests —race 56			All			
Egypt Na95 × Marquis F_2						
Seedling tests —race 15B	Observed		59	26		
	Expected		63.8	21.2	3 : 1	.20–.30
Seedling tests —race 56	Observed		68	18		
	Expected		64.5	21.5	3 : 1	.30–.50
Egypt Na95 × Kenya 117A F_2						
Seedling tests —race 15B	Observed		137	0		
	Expected		137	0		
Seedling tests —race 56	Observed		130	0		
	Expected		130	0		
Field tests —race 56	Resistant—0–3% rust (996 plants)					
Egypt Na95 × Kenya 58 F_2						
Seedling tests —race 15B	Observed	104	32	0		
	Expected	102	34	0	3 : 1	.70–.80
Seedling tests —race 56	Observed	102	22	4		
	Expected	96	30	2	48 : 15 : 1	.10–.20
Field tests —race 56	Segregated—0–80% rust (1225 plants)					
Egypt Na95 × Red Egyptian F_2						
Seedling tests —race 15B	Observed	105	33	3		
	Expected	105.8	33.0	2.2	48 : 15 : 1	.80–.90
Seedling tests —race 56	Observed		138	0		
Field tests —race 56	0–50% rust (1323 plants)					

very good fit to a ratio of 3 moderately resistant plants : 1 susceptible. This anomalous result cannot be explained unless the segregation of 12 segregating : 30 susceptible families is an extremely poor fit to a 1 : 1 ratio.

The backcross families derived from the remaining three F_1 plants behaved differently. When tested with race 56, half of this second group of families segregated 3 moderately resistant plants : 1 susceptible and approximately one quarter segregated 15 moderately resistant plants : 1 susceptible. Evidently two genes for resistance to race 56 were segregating. It seems probable that only one of these two genes was present in the first group of families. When the second group of families was tested with race 15B, 25, or exactly half, segregated for resistance. In a few families the classification of the plants was difficult and as a result the combined segregation within families was not a good fit to a ratio of 3 moderately resistant plants : 1 susceptible. However, it seems likely that a single, partially dominant gene conditions resistance to race 15B. The type of resistance segregating in these families appeared identical to that present in the 12 segregating families in the first group. On the basis of the backcross results it is considered probable that Egypt Na95 normally carries one gene for resistance to race 15B and two for resistance to race 56.

With one exception, the results of tests on F_2 populations from the various crosses involving Egypt Na95 agree with the above hypothesis. The exception occurred in the cross Egypt Na95 × Marquis where only one gene for resistance to race 56 appeared to be segregating.

Further proof of the hypothesis comes from the tests on the cross Egypt Na95 × Kenya 117A. All plants of the F_2 population were resistant to both races. Kenya 117A has already been shown to have only one gene, *Sr7*, for resistance to race 15B and two genes, *Sr10* and probably *Sr9*, for resistance to race 56. Egypt Na95 must, therefore, carry *Sr7* and either one or both of *Sr9* and *Sr10*. The types of resistance to race 56 conditioned by the two genes which are present in most of the Egypt Na95 plants, appeared identical to the types of resistance conditioned by *Sr9* and *Sr10*. It is probable, therefore, that Egypt Na95 and Kenya 117A have both genes in common. Final proof of this will require the separation from each variety of its two genes for resistance to race 56, followed by intercrossing of the derived lines.

The presence of *Sr7* in Egypt Na95 is further demonstrated by the fact that all F_2 plants from the cross Egypt Na95 × Kenya 58 were either very resistant or moderately resistant to race 15B. As was the case in the cross Kenya 117A × Kenya 58, *Sr6* behaved as a dominant gene and three fourths of the plants gave a fleck reaction to race 15B. For resistance to race 56, three genes, *Sr6* from Kenya 58 and *Sr9* and *Sr10* from Egypt Na95, were segregating and the expected ratio of 48 very resistant plants : 15 moderately resistant : 1 susceptible was obtained.

The cross Egypt Na95 × Red Egyptian segregated for resistance to race 15B. Three genes were present, *Sr6* and *Sr8* from Red Egyptian and

TABLE 11.—RESULTS OF RUST TESTS ON F_2 SEEDLINGS FROM CROSSES INVOLVING MCMURACHY

Variety or cross and race of rust		Number of plants			Ratio	P
		[VR	[MR	[S		
McMurachy						
Race 15B		All				
Race 56		All				
McMurachy × *Kenya 58 F₂*						
Race 15B	Observed	150	0	0		
	Expected	150	0	0		
Race 56	Observed	149	0	0		
	Expected	149	0	0		
McMurachy × *Red Egyptian F₂*						
Race 15B	Observed	128	0	0		
	Expected	128	0	0		
Race 56	Observed	125	0	0		
	Expected	125	0	0		
McMurachy × *Kenya 117A F₂*						
Race 15B	Observed	102	33	9		
	Expected	108	27	9	12 : 3 : 1	.30–.50
Race 56	Observed	138		2		
	Expected	137.8		2.2	63 : 1	1.0
McMurachy × *Egypt Na95 F₂*						
Race 15B	Observed	35	12	5		
	Expected	39	9.8	3.2	12 : 3 : 1	.50–.70
Race 56	Observed	49		2		
	Expected	50.2		.8	63 : 1	.30–.50

Sr7 from Egypt Na95, and a very good fit to the expected ratio of 48 very resistant seedlings : 15 moderately resistant : 1 susceptible was obtained. The hypersensitive reaction to race 15B conditioned by *Sr6* was clearly dominant. With race 56, 138 seedlings were tested and none was more susceptible than type 2. In the field 1323 plants were tested with race 56 and although 7 plants carried 50 per cent rust, none was as susceptible as Marquis. The results are consistent with the hypothesis that Egypt Na95 carries gene *Sr9* in common with Red Egyptian.

McMurachy

McMurachy was not included in the diallel crosses but was crossed with the four varieties which are resistant to race 15B. The results of seedling tests on F_2 populations from the McMurachy crosses are given in Table 11.

The data show that McMurachy carries gene *Sr6* in common with Kenya 58 and Red Egyptian. The F_2 seedlings from the crosses McMurachy × Kenya 58 and McMurachy × Red Egyptian exhibited a hypersensitive fleck reaction to both races 15B and 56. That *Sr6* is the only gene for resistance to races 15B and 56 carried by McMurachy is shown in the crosses with Kenya 117A and Egypt Na95. With race 15B, the F_2 populations from both crosses segregated in a ratio of 12 very resistant seedlings : 3 moderately resistant : 1 susceptible, indicating that each variety contributed only one gene. The gene *Sr6* again behaved as a dominant for resistance to race 15B. With race 56, a ratio of 63 resistant seedlings : 1 susceptible was obtained indicating the presence of three genes for resistance. Since Kenya 117A and Egypt Na95 each have two genes, only one can have come from McMurachy.

Gabo, Lee and Timstein

The three varieties Gabo, Lee and Timstein are very similar in both their seedling and mature plant reactions to race 56. All three give type $1\text{–}1^+$ pustules in the seedling stage and show a variable percentage of moderately resistant type pustules under field conditions (Table 12).

TABLE 12.—RESULTS OF FIELD TESTS WITH RACE 56 ON THE PARENTS AND F_2 POPULATIONS OF THE CROSSES GABO × LEE, GABO × TIMSTEIN AND LEE × TIMSTEIN

Variety or cross	Number of plants with the following percentage rust[1]										
	O	T	1	3	5	10	20	30	40	50	Total
Gabo	15	46	95	25	63	31	11	4	1		291
Lee	12	58	288	83	120	40	8	1	1		611
Timstein	6	61	103	50	79	44	15	1	1	1	361
Gabo × Lee	14	22	134	114	282	176	74	10	3		829
Gabo × Timstein	29	1	91	88	150	94	33	3			489
Lee × Timstein	10	168	544	173	86	19	2				1002

[1] The pustules on both the varieties and their hybrids were of a semi-resistant type.

TABLE 13.—PROBABLE GENOTYPE OF EACH VARIETY

Variety	Genotype					
Kenya 58	*Sr6Sr6*	*Sr7Sr7*				
Red Egyptian	*Sr6Sr6*		*Sr8Sr8*	*Sr9Sr9*		
Kenya 117A		*Sr7Sr7*		*S9gSr9*	*Sr10Sr10*	
Egypt Na95		*Sr7Sr7*		*Sr9Sr9*	*Sr10Sr10*	
McMurachy	*Sr6Sr6*					
Gabo					*Sr11Sr11*	*Sr12Sr12*
Lee					*Sr11Sr11*	*Sr12Sr12*
Timstein					*Sr11Sr11*	*Sr12Sr12*

TABLE 14.—GENE EXPRESSION AND THE RUST REACTION OF HOMOZYGOUS PLANTS.

Gene	Race	Gene expression	Reaction of homozygotes	
			Seedlings	Mature plants
Sr6	15B	Recessive in some crosses, dominant in others	;	R
	56	Dominant in seedlings, partially dominant in mature plants	;	R
Sr7	15B	Partially dominant	1–1+	MR
	56	No effect	4	S
Sr8	15B	Partially dominant	2–2+	MS
	56	Partially dominant	2–2+	—
Sr9	15B	No effect	4	S
	56	Partially dominant	2–2+	MS
Sr10	15B	No effect	4	S
	56	Partially dominant	1+–2	R
Sr11Sr12,	15B	No effect	3+	S
	56	Complementary and dominant	1–1+	MR

The results of field tests with race 56 on F_2 populations from the crosses between Gabo, Lee and Timstein are shown in Table 12. All plants from the three crosses fell within the range of rust percentages recorded for the parents indicating that the three varieties have in common a gene or genes for resistance to race 56. As noted in the Review of Literature, Timstein and Lee have been shown to have two dominant complementary genes on chromosome X. The same two genes must be present in Gabo. Since symbols have not been given to these two genes, they are tentatively listed as *Sr11* and *Sr12* following the system used in this paper.

The crosses Gabo × Marquis and Timstein × Marquis produced only sterile, dwarf plants and no backcrosses were possible. There is no doubt, however, that the two genes on chromosome X are the only genes for resistance to race 56 carried by Gabo and Timstein. A few backcrosses of Lee to Marquis were tested with race 56 and no evidence was found for additional genes for seedling resistance in Lee. All of the crosses of Gabo, Lee and Timstein with the remaining varieties segregated for resistance to race 56.

Thatcher

No genetic analysis of Thatcher was attempted. However, results from 14 F_2 families from a backcross to Marquis suggest that at least 3 genes are involved in resistance to race 56. Thatcher did not have a gene or genes in common with any of the other varieties studied.

A complete summary of the genetic analyses from the preceding pages is given in Tables 13 and 14. The genotype of each variety is listed in Table 13 and the genes are described in Table 14.

DISCUSSION

In the work reported in this paper an attempt was made not only to determine the mode of inheritance of rust resistance but also to determine the interrelationships of the genes in the varieties studied. Generally the results agree with those obtained for the same varieties by other authors. It was not possible, however, to relate the present work with races 15B and 56 to work which has been done with other races.

In the present study the three varieties Kenya 58, McMurachy and Red Egyptian have been shown to carry a gene, *Sr6*, which conditions a hypersensitive type of resistance to races 15B and 56. Peterson *et al.* (see Goulden, 4) and Shebeski (16) had previously reported that McMurachy and Red Egyptian have a gene in common. Peterson and Campbell* and Sears and Rodenhiser (as reported by Sears, 14) located this gene on chromosome XX. Red Egyptian has been shown in the present study to carry two additional genes, *Sr8* which conditions moderate resistance to both races 15B and race 56, and *Sr9* which conditions moderate resistance to race 56. This last gene is probably also present in Kenya 117A and Egypt Na95. As reported by Sears (14), Sears and Rodenhiser located one gene in Red Egyptian on chromosome VI. This gene provides resistance to both races 15B and 56 and corresponds to *Sr8*. Sears* and Loegering located on chromosome XIII in Red Egyptian, a gene which provides resistance to race 56 and corresponds to *Sr9*. In this paper both Kenya 58 and Kenya 117A have been shown to carry a gene, *Sr7*, which provides resistance to race 15B. Athwal and Watson (1) reported that Kenya 117A carries two genes for stem rust resistance but it is not possible to tell whether they are the same as two of the three genes reported here.

The results given in this paper indicate that Kenya 58 carries two genes for resistance to race 15B. This is not in agreement with the work of Hasanain (5) who concluded that Kenya 58 has three genes, equal and cumulative in effect.

*Personal communications.

As noted earlier, Gabo was found to carry the same two complementary dominant genes for resistance to race 56 that are present in Timstein and Lee. Sears and Rodenhiser (15) and Plessers (12) located these two genes on chromosome X.

The discovery that Gabo carried the same resistance as Timstein was unexpected since Gabo derived its resistance from Gaza durum while Timstein obtained its resistance from *Triticum timopheevi* Zhukov. It was noted, however, that Gabo and Timstein were very similar in appearance and had factors in common for leaf and stem rust resistance, winter growth habit and dwarfness. The similarity seemed too great to be a coincidence and it was concluded that the two were probably sib lines from the same cross. Watson* has concluded on the basis of tests with leaf and stem rust that the Timstein that has been distributed in North America is not the true variety. Apparently, the same stock of Timstein that was used in the crosses reported in this paper was also studied by Sears and Rodenhiser (15) and was one of the parents of Lee. Koo and Ausemus (8) may, however, have had the true variety since their results show the presence of only one gene for resistance rather than two.

In the course of this study a number of conclusions were reached in regard to methods for genetic analysis of rust resistance. It was found that the study of F_2 families from backcrosses to a susceptible variety has advantages over the study of F_2 lines. In backcrosses the ratios are simpler and it is easier to separate genes for resistance and study their effects singly. Although in many cases good ratios are obtained from studies of segregations within families, ratios involving backcross families rather than individual plants are more dependable.

Field studies proved to be less valuable than seedling studies. In the field many factors such as diseases, spacing, moisture, weather and maturity influence the rust reaction of individual plants so that their correct classification is often difficult or impossible. Plants in a segregating population usually show a continuous variation from resistance to susceptibility. However, field tests are desirable to compare seedling and mature plant reactions and to test for the presence of genes providing only mature plant resistance. In the varieties reported in this paper no genes for mature plant resistance were found. It was noted, however, that in several cases genes were less dominant in mature plants than in seedlings. For example, plants heterozygous for *Sr6* gave a hypersensitive reaction to race 56 as seedlings but in the field showed only moderate resistance to the same race. Similarly, plants heterozygous for the two complementary genes from Gabo gave a type 1^+ reaction to race 56 as seedlings but appeared susceptible to the same race in the field.

From a plant breeding point of view it was interesting to find that genes which by themselves provided resistance only to race 56 acted as modifiers of resistance to race 15B. Thus backcross families segregating for only one gene, such as *Sr7*, showed less field resistance to race 15B than families segregating for *Sr7* plus genes for resistance to race 56, such as *Sr9* and *Sr10*. The occurrence of this type of modifying action may account for the difficulty that has sometimes been encountered in crosses and

* Personal communication.

backcrosses in fully recovering rust resistance that seemed to be simply inherited. It is, perhaps, not too surprising to find genes for resistance to race 56 having an effect on race 15B. In this study two cases of a single gene giving resistance to both races have been found and this suggests that the resistances to races 15B and 56 are closely related physiologically. The results indicate the desirability of combining a number of sources of resistance in a single variety.

A weakness in the present study lies in the fact that only two races of rust were used. Races 15B and 56 were selected because they represent two distinct groups of rust races. However, genes which provide resistance to races other than those studied were not detected. Furthermore, no idea was obtained of the number of races to which the known genes provide resistance. Further work is in progress to give information on this last point. Each gene that is discovered is being transferred to Marquis by backcrossing. When the backcrossing is completed the derived lines will be compared to the original Marquis for resistance to a considerable number of races.

The program to determine the interrelationships of the genes for rust resistance carried by various varieties is being continued and extended.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to L. H. Shebeski and B. C. Jenkins, Plant Science Department, University of Manitoba, for their assistance, particularly in the initial planning of this work.

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EFFECTS OF FIELD EXPOSURE ON IMMATURE STAGES OF THE WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORT. (HYMENOPTERA: CEPHIDAE)[1]

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[Received for publication November 24, 1955]

ABSTRACT

Effective control of the wheat stem sawfly, *Cephus cinctus* Nort., is obtained by exposing the stubs on the surface of the soil at any time in the fall. Because of the danger of reinstating diapause, spring exposure should be limited to the latter part of May and the early part of June, when most sawflies are in the prepupal and pupal stages. If exposure occurs too late, adults will emerge.

INTRODUCTION

The soil around the wheat stubs in which larvae of the wheat stem sawfly, *Cephus cinctus* Nort., hibernate provides excellent insulation against extremes of temperature. Records at Lethbridge, Alberta, show that the temperature 1 inch below the surface of the soil ranges from 23° F. in the winter to 86° F. in the summer.

Farstad (2) recommended shallow cultivation in the spring or fall to destroy sawflies by exposing the infested stubs on the surface of the soil. Salt (3, 4) found that larvae of *C. cinctus* died after they had lost over 40 per cent of their body weight through desiccation and that susceptibility to desiccation increased progressively from the larval to the adult stage. Salt stated that precipitation during exposure might negate the effects of desiccation.

As reported by Salt (5), Farstad found that some larvae returned to diapause when the stubs were exposed by spring tillage or when hot dry conditions existed in the spring. In these cases, many of the larvae would withstand unfavourable conditions during the summer and would emerge the following spring. Following this lead, Salt (5) showed that, at 35° C., development was prohibited; prepupae and pupae were deformed, and non-diapause larvae re-entered diapause. Later, Church (1) confirmed that high temperature was the main factor in reinstatement of diapause and showed that desiccation halted larval development but did not reinstate diapause.

The present paper deals with the development, diapause, and mortality of *C. cinctus* in stubs exposed on the soil surface and shows the relationship between time of exposure and its efficiency as a control practice.

SPRING EXPOSURE

Experimental Conditions

This experiment was conducted in 1948 at the Canada Experimental Sub-station, Regina, Saskatchewan. Between May 9 and June 4, stubs were dug up at intervals of 2 to 4 days and placed on the surface of the soil,

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TABLE 1.—PERCENTAGES OF *C. cinctus* IN VARIOUS STAGES AT THE BEGINNING OF EACH SPRING EXPOSURE[1]

Date exposed	Larvae	Prepupae	Pupae	Adults	Dead[2]
May 9	91	2	0	0	7
May 11	89	2	0	0	9
May 14	90	0	0	0	10
May 17	92	1	0	0	7
May 21	91	0	0	0	9
May 25	51	33	10	0	6
May 28	6	52	29	0	13
June 1	11	18	63	0	8
June 4	3	7	80	5	5

[1] From 100 stubs for each date.
[2] Including parasitized larvae.

where they were held in place with strips of stucco wire. In addition, 200 stubs were dug up and exposed on June 19. Each treatment was replicated four times. One plot in each replicate was left undisturbed as a check. Random samples of 25 stubs from each plot were split on the first day of exposure and regularly thereafter. On July 9, 100 stubs from each plot were split; the remainder were stored at approximately 0°C. to break the second period of diapause of the surviving larvae.

Maximum daily temperatures on the surface of the plots varied from 75° to 120° F. (mean 100° F.) during the exposure period. The mean maximum daily air temperature for May was 73° F. (range 53° to 90° F.) and precipitation was 0·03 inches. In June, the mean maximum daily air temperature was 74° F. (range 59° to 93° F.) and precipitation was 2·01 inches.

The stage of development of the sawfly population for each date of exposure is given in Table 1. Until May 28 most individuals were larvae or prepupae; the first pupae were observed on May 25 and by June 4 only 3 per cent of the population were larvae.

Results and Discussion

Figure 1 shows that the date of exposure was more important than the duration of exposure. Larval survival decreased progressively from 35 per cent for the May 9 exposure to 1 per cent for the June 4 exposure. Over 90 per cent of the sawflies exposed between May 25 and June 4 died; most of these were in the prepupal or pupal stages at the time they were first exposed (Table 1). Seventeen per cent of the sawflies exposed on June 19 were sufficiently developed to emerge as adults before they could be killed by the exposure.

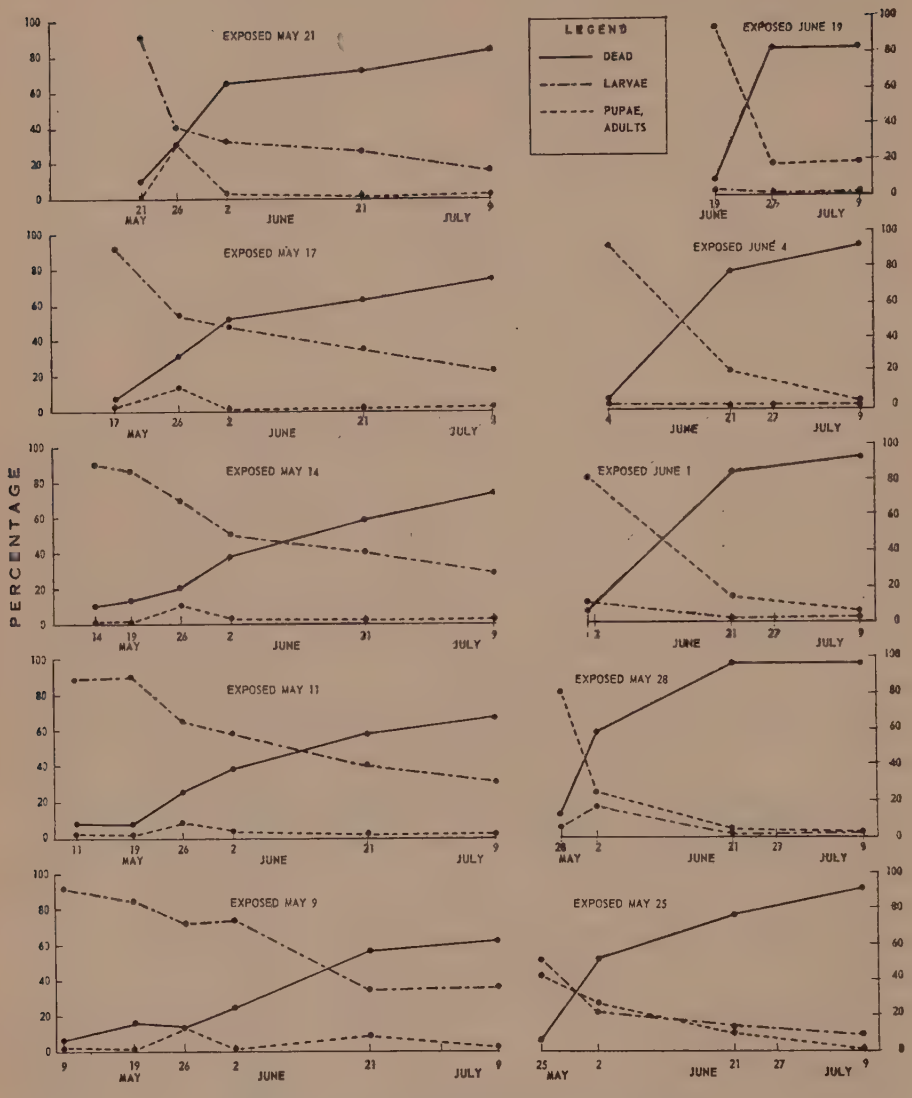

FIGURE 1. Percentages of *C. cinctus* dead and in various stages on various dates after exposure on different dates in the spring.

Most of the individuals that died were in the post-larval stages (prepupae are included as pupae in Figure 1). The highest rate of mortality for the exposures of May 9 to 21 occurred shortly after May 26, when most individuals that failed to re-enter diapause had developed to the prepupal or pupal stage. Only a relatively short period of exposure without excessively high temperatures and even with some precipitation was sufficient to cause considerable mortality among the post-larval stages. Within 5 days 45 per cent of the sawflies exposed on May 28 died. During this period no precipitation occurred and the temperatures were:—

Date	Daily maximum temperature on soil surface °F.	Daily maximum air temperature °F.
May 28	104	76
29	98	74
30	120	81
31	116	83
June 1	108	93

Seventy-five per cent of the sawflies exposed on June 19 were dead by June 27. Temperatures and precipitation recorded for this period were:—

Date	Daily maximum temperature on soil surface, °F.	Daily maximum air temperature, °F.	Precipitation, in.
June 20	108	69	trace
21	80	69	0
22	92	73	0
23	92	78	0·06
24	100	64	0·67
25	90	66	0·01
26	88	74	0·08

Weather conditions causing high mortality from exposure occurred generally throughout the exposure period. The greatest increase in mortality occurred between June 2 and June 21 for sawflies exposed on May 9 and 11, between May 26 and June 2 for those exposed on May 14, and by June 2 for those exposed from May 17 to May 28.

Most of the larvae that survived exposure emerged as adults after the subsequent cold treatment. However, the body and wing lengths of these adults were less than those of normally emerged adults. This suggests that nutrients utilized for the extra period of diapause were used at the expense of production of adult tissues.

These results agree closely with those reported by Salt (3, 4). However, because his experiments were conducted at 40° C. and 0 per cent relative humidity, no adults emerged and diapause was not reinstated. Under field conditions, fluctuating temperatures and humidities permitted some individuals to re-enter diapause and others to complete their development.

Church (1) reported that 1 week at 95° F. usually caused all larvae to return to diapause, and 2 days at the same temperature caused 19 to 28 per cent of the larvae to return to diapause. Because 29 and 15 per cent of the larvae exposed between May 14 and 21 re-entered diapause, they

were probably subjected to an equivalent of 2 days at 95° F. during the early part of the exposure period. It appears that temperature was the main factor operating during the first part of the exposure period but whether high temperature or desiccation was the major factor in production of mortality is not known. Salt (3) found that temperatures up to 104° F. alone did not have lethal effects, and that resistance to desiccation decreased progressively from the larval to the adult stage. The data for the period from June 20 to 26, when approximately 75 per cent of the individuals died and when the maximum temperature on the surface of the soil rose above 104° F. on only 1 day, suggest that desiccation was the major factor, although considerable rain did fall during the period.

Under normal field conditions, chances for survival of the larvae that re-entered diapause until the following spring would be good. Because of the necessity of controlling weeds, further tillage operations after the initial exposure would probably bury most of the stubs and so provide the larvae with ample protection from the weather. Such operations would be done well before July 9, the end of the exposure period in this test.

Conclusions

The data show the importance of proper timing of spring exposure to control the wheat stem sawfly. Highest mortalities were obtained in stubs exposed when most individuals were in the prepupal and pupal stages. This period usually occurs in late May and early June. Progressively earlier exposures permitted increased numbers of larvae to survive the effects of exposure by re-entering diapause.

FALL AND WINTER EXPOSURE

Experimental Conditions

Two thousand stubs were exposed on the surface of the soil on each of 7 dates during August and September, 1948, at Lethbridge, Alberta. Stubs of both winter and spring wheat were used. One hundred stubs per treatment were split and examined at weekly intervals until October 1. The remainder were left on the plots during the winter and were examined on June 3, 1949.

No precipitation was recorded from August 14 to October 1 and the mean maximum temperature on the soil surface during the period was 100° F. (range 74° to 127° F.).

Results and Discussion

The comparatively high percentage of larval mortality (Table 2) in the first-exposed stubs probably resulted from the high temperature (127° F.) on August 14, when many of the larvae in these stubs had just cut the stems and had not yet secreted their cocoons. There was little difference in mortality among the plots exposed after August 14. Salt (3) found a progressive increase in resistance to desiccation during August followed by an approximately equal decrease during September. Except for the first date of exposure, such an effect was not observed in this experiment.

TABLE 2.—PERCENTAGE OF *C. cinctus* DEAD ON VARIOUS DATES AFTER EXPOSURE ON DIFFERENT DATES IN THE FALL OF 1948

Date exposed	Host[1]	Weeks after exposure								On June 3, 1949
		0	1	2	3	4	5	6	7	
Aug. 14	w.w.	5	41	47	50	53	58	60	60	100
Aug. 20	w.w.	5	10	11	20	22	23	22	—	100
Aug. 27	w.w.	7	15	19	23	21	24	—	—	100
Sept. 1	s.w.	5	5	18	19	28	—	—	—	98
Sept. 2	w.w.	9	13	21	17	24	24	—	—	100
Sept. 8	s.w.	4	21	21	—	—	—	—	—	100
Sept. 29	s.w.	6	—	—	—	—	—	—	—	100

[1] w.w., winter wheat; s.w., spring wheat.

Neither time of exposure nor host variety had a differential effect on survival of the larvae exposed in stubs over the winter.

Salt (6) reported that mature larvae of *C. cinctus* have undercooling points lying mostly between −4° F. and −20° F. During the exposure period the temperature fell below −20° F. on 19 days. From May 9 to July 9 at Regina in 1948, the total precipitation was 2.07 inches, there were 22 days on which the air temperature rose above 75° F., and 35 per cent of the larvae exposed during this period survived. From April 1 to June 3, 1949, at Lethbridge, 5.56 inches of precipitation were recorded, the air temperature exceeded 75° F. on 14 days, and, except for 2 per cent of the larvae exposed on September 1, all the individuals examined on June 3 were dead larvae. Although the weather conditions during the spring of 1949 were more favourable for survival than during the spring of 1948, larval survival was extremely low for those exposed in the fall of 1948; therefore it appears conclusive that most if not all of the mortality occurred during the winter.

Conclusions

Although the weather conditions during the winter of 1948-1949 at Lethbridge were not unusually severe, excellent control of exposed larvae was obtained. One may conclude that most larvae of the wheat stem sawfly exposed on the surface of the soil at any time during the fall stand little chance of surviving to the adult stage.

ACKNOWLEDGEMENTS

The writers wish to thank R. W. Salt, L. K. Peterson and other officers of the Lethbridge Laboratory, for their advice and assistance.

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DOSAGE DES CATIONS ÉCHANGEABLES DU SOL PAR LE SPECTROPHOTOMÈTRE À FLAMME BECKMAN

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[Received for publication December 20, 1955]

RÉSUMÉ

L'auteur préconise une technique pour éliminer (avec le spectrophotomètre à flamme de Beckman, Modèle DU) l'obstruction du brûleur (clogging) dans le dosage direct des bases extraites par une solution d'acétate d'ammonium. Cette solution est diluée, moitié-moitié, avec de l'eau distillée contenant 10 pour cent d'alcool éthylique et 0·5 pour cent de triéthanolamine (solution de Dmitrieff-Kokline). Six mille dosages ont été effectués avec le même brûleur sans aucune obstruction.

L'extraction des bases échangeables se fait de plus en plus par une solution neutre et normale d'acétate d'ammonium. C'est la méthode officielle de "Association of Official Agricultural Chemists".

Pour les analyses de routine, le dosage direct des cations Ca, Mg, K, Na, dans la solution d'acétate d'ammonium par le photomètre à flamme serait une économie appréciable de temps. Cependant, l'obstruction très lente du brûleur cause des fluctuations dans les lectures obtenues.

Cette obstruction lente du brûleur (clogging) a été constatée et décrite par plusieurs auteurs. Natelson (3) constate cette obstruction dans l'analyse du sang et de l'urine par l'appareil Perkin-Elmer, et préconise un brûleur d'une ouverture plus grande. En 1951, Fieldes *et al.* (2) éprouvèrent les mêmes ennuis avec une solution d'acétate d'ammonium dans le dosage des bases échangeables. Ces auteurs ont utilisé un autre type de brûleur. La sensibilité obtenue semble insuffisante pour le dosage du calcium et du magnésium. De plus, d'après les mêmes auteurs, la silice précipitée est la cause principale de l'obstruction.

Le même phénomène d'obstruction a été constaté par Rich (4) en 1952. Il recommande, pour obvier à cet inconvénient, de centrifuger la solution à doser.

Une autre cause de cette obstruction semble être le carbone qui se dépose à l'orifice du brûleur.

Dans le but de diminuer cette obstruction, on expérimenta une solution préconisée par Dmitrieff-Kokline (1). Cette solution se compose d'eau triplement distillée contenant 10 pour cent d'alcool éthylique et 0·5 pour cent de triéthanolamine. D'après cet auteur, cette solution est "mouillante, plastifiante et alcaline", ce qui évite la détérioration du brûleur.

En premier lieu, on évapora à sec la solution d'acétate d'ammonium contenant les bases échangeables, et on essaya de dissoudre le résidu dans la solution alcoolique de Dmitrieff-Kokline. Les résultats indiquent clairement que la solubilité des acétates de calcium et de magnésium est très incomplète dans cette solution alcoolique. On expérimenta alors, la technique suivante, et elle donna entière satisfaction. Six mille dosages ont été effectués avec le même brûleur sans aucune obstruction et sans dépôt de carbone à l'orifice du brûleur.

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TECHNIQUE SUIVIE

On extrait, par une solution normale d'acétate d'ammonium, pH 7·0, les bases échangeables contenues dans 25 gm. de sol non-moulu et tamisé sur tamis de 2 mm. On complète le volume à 250 ml.

Avant de lire au spectrophotomètre de Beckman (Model DU, avec photomultiplicateur), on dilue moitié-moitié la solution d'acétate d'ammonium contenant les bases échangeables avec la solution alcoolique décrite plus haut.

Comme solutions standards, on utilise des solutions dont le solvant est un mélange à parts égales de la solution normale d'acétate d'ammonium et de la solution alcoolique.

Chaque solution standard contient du calcium, du magnésium, du potassium et du sodium. Le point 100 du spectrophotomètre à flamme correspond à 12 p.p.m. de Na, à 48 p.p.m. de K, à 120 p.p.m. de Mg et à 300 p.p.m. de Ca. Les points inférieurs à 100 correspondent à 23 autres solutions contenant les mêmes éléments en progression arithmétique décroissante.

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AN APPARATUS FOR REMOVING PUPARIA AND LARVAE FROM SOIL[1]

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[Received for publication December 9, 1955]

ABSTRACT

The apparatus was designed to separate puparia from soil. The operation involves washing infested soil through two screens of different meshes. The lower mesh screen is manually vibrated and brushes are moved across the upper screen to break soil masses. The upper screen removes large debris, and the lower one retains the puparia and debris of similar size. Puparia are floated off in clear water and collected with a piece of screen wire. Larvae must be picked from the debris. The apparatus serves equally well for wet or dry soil and has been shown to remove all *Hylemya brassicae* (Bouché) pupae added to soils of different types.

INTRODUCTION

Collecting soil-inhabiting pupae and larvae, especially the smaller species, is often a slow and laborious operation. Of the many types of apparatus described for this type of work, the one devised by Lafrance (1) has been widely used, but only with relatively dry soil. During the drying, puparia become dull brown in colour and particles of soil adhere to them so that it is difficult to separate them from the debris. In root maggot investigations at the Charlottetown Laboratory, a method was devised for rapid separation of puparia from either wet or dry soil. This apparatus is described herein.

DESCRIPTION OF APPARATUS

The apparatus (Figures 1 and 2) consists of a large water tank (G) with a false bottom (H), two screen trays (C and D), a screen tray holder (E) fitted with a manually operated vibrator (F), a galvanized iron tray (I), and a common type of shower nozzle (A) fitted to a garden hose.

The tank, tray, and false bottom are made of heavy-gauge galvanized iron. The false bottom is reinforced with 1 × $\frac{1}{4}$-inch flat-iron braces riveted to it (Figure 2, H). The dimensions are as follows: tank, 30 × 26 × 4 inches; tray, 30 × 20 × 6 inches; and false bottom, 29 × 26 inches.

The upper (C) and lower (D) screen trays are made of $\frac{3}{4}$-inch dressed pine with wire screening on the bottom. Other specifications are: upper tray, 25 × 18 × 7 inches with 4-mesh screening; lower tray, 26 × 19 × 7 inches with 16-mesh screening. Around the inside of the lower tray is a $\frac{1}{2}$-inch rabbet in which the upper tray rests (Figure 1). Three strands of No. 9 wire are stretched across the bottom of each tray to reinforce the screening.

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The screen tray holder (E) and the vibrator (F) are made of $\frac{3}{4}$-inch dressed pine and fit the screen trays and the tank (Figure 1). and the vibrator is hinged to the tray holder. The bars on the vibrator ($10 \times \frac{3}{4} \times \frac{3}{4}$ inches) are nailed to the vibrator and are so arranged that they lie between and parallel with the reinforcing wires on the bottom of the screen tray when the apparatus is in operation.

Stiff fibre-bristled brushes (B) are mounted on 1-inch pine fitted to the upper tray (C, Figure 1) and are so arranged that in operation the bristle tips are a half-inch above the screen.

METHOD OF OPERATION

Collecting Puparia

Figure 1 shows the apparatus as set up for operation. About one peck of wet or dry soil is placed in the upper screen tray and washed with a stream of water from the spray nozzle. High pressures of water may be used without danger of injuring the puparia. When the soil becomes saturated, it is stirred by sliding the brushes quickly from side to side, thus breaking up lumps and keeping the soil moving through the screen. If two or three pecks of soil are being washed through at one time, the brushes cannot be used but the operation can be greatly speeded up by stirring by hand; rubber gloves are advisable for this operation. Throughout the washing, the lower screen is tapped by an up-and-down motion of the handle of the vibrator to prevent clogging of this screen and accumulation of water in the tray.

When the washing on the upper screen is completed, this tray is removed and the water allowed to spray directly into the lower tray. Materials that have accumulated on the lower screen are agitated by constant movement of the vibrator arm and the washing is continued until all colloidal material and particles smaller than the openings of the screen have been washed through. The lower tray (Figure 2, D), which retains the puparia and similar-sized particles of debris, is then placed in the galvanized iron tray (I). Water is added until the level is about 2 inches above the wire screening. Puparia float to the surface and are easily removed with a piece of spoon-shaped fine wire screening or the strainer from a potato spray nozzle fitted with a short handle.

The water that collects in the tank is carried off by means of a rubber hose (Figure 2, J) and periodically the soil that settles to the bottom of the tank is removed by lifting out the false bottom.

Collecting Larvae

The apparatus may be used for removing many kinds of insect larvae from the soil. Screens of different meshes are used, depending on the size of the larvae to be collected. The upper screen should have openings of about the same size as the length of the larvae, and the openings in the lower screen should be just small enough so that the larvae will not pass through.

In washing the soil through the screens the same procedure as outlined for collecting puparia is followed. However, to avoid injuring the larvae, it is advisable to use a low pressure of water in the washing hose. When the soil is removed, the larvae are retained on the lower screen.

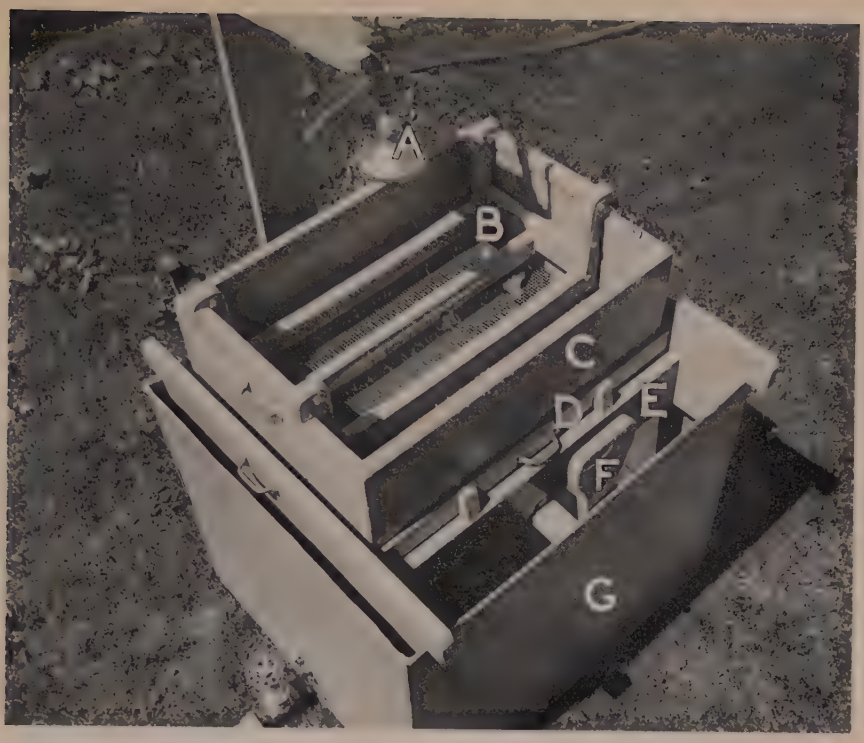

FIGURE 1. Apparatus for removing puparia from soil, set up for operation.

FIGURE 2. Apparatus for removing puparia from soil, dismantled to show construction details.

As the larvae of most insects do not float in water, the floating tray is not used in the manner described for puparia. Instead, the screen tray containing the larvae is dipped in and out of the tray of water several times and then removed. The larvae, together with lighter debris, rise toward the surface and are picked out with forceps.

DISCUSSION

The apparatus has been used mainly for collecting puparia of *Hylemya brassicae* (Bouché), *H. liturata* (Mg.) (=*H. trichodactyla* (Rond.)), *H. cilicrura* (Rond.), *Coenosia tigrina* (Fall.), and *Muscina stabulans* (Fall.), and also for obtaining mature larvae of *H. brassicae* from soil. In tests with 100 puparia of *H. brassicae* in different types of soil, 100 per cent were recovered in every case. Flies emerged from approximately 95 per cent of the puparia, indicating that the washing procedure did not injure the insects.

This apparatus can be used with soil samples, wet or dry, taken directly from the field. During the washing, puparia are shiny brown in colour and are easily distinguished from any floating debris in the water. The operation of removing the puparia from a pailful of soil taken directly from the field requires about 15 minutes and, except for conveying the soil to the machine, little manual labour is required.

ACKNOWLEDGEMENTS

The helpful suggestions of F. M. Cannon, Officer-in-Charge of the Charlottetown Laboratory, in designing the apparatus, are gratefully acknowledged.

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A ROLLER THRESHER FOR EXPERIMENTAL PLOT MATERIAL[1]

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[Received for publication June 9, 1955]

ABSTRACT

A small thresher for experimental plot material, using the roller principle as applied in fibre flax mills, was designed and built at the Winnipeg laboratory. Details of construction and operation are given. This thresher has a number of features that are advantageous in threshing small plot samples. Mechanical damage to the seed is reduced significantly; mixing of seed of different varieties in the threshing operation is eliminated completely; it is not necessary to clean the machine between varieties; and small or light kernels are retained giving a closer approximation to the true yield. The roller thresher has proven satisfactory for threshing experimental plots of wheat, oats, barley, flax, soybeans, radishes, onions, alfalfa and rapeseed.

INTRODUCTION

In plant breeding the need of threshing large numbers of individual plants or small sheaves has led to the designing of specialized miniature threshing machines. Most of these employ a small scale threshing cylinder comparable to the larger cylinders used in farm threshers. In using such machines it is difficult to eliminate the following defects in operation:

1. Mechanical damage to the seed.
2. Lodging of seed in the machine leading to mixing of varieties.
3. Loss of a proportion of the seed.
4. Varying efficiency of operation with crops of different seed sizes.

The roller thresher described here was developed in an attempt to eliminate these difficulties.

The principle used in the roller thresher has been employed previously for a variety of purposes. For example, fibre flax mills have used rolls of metal or other materials to remove seed bolls from flax straw, and the Minnesota Agricultural Experiment Station has used a pair of pulleys as rolls, one of which is driven by power and the second by friction on the first roll, to thresh experimental linseed flax plots. Because of the bulky nature of these machines developmental work on a more compact machine, using the roller principle, was begun at this laboratory in 1952.

The first model developed at Winnipeg had rolls 8 inches in diameter and 18 inches in length with only one roll driven by power. This machine was satisfactory for single plants or quite small sheaves of flax or cereals, but the free roll often failed to turn if a sheaf from a 10-foot drill was being threshed. Another model was therefore built with both rolls 14 inches in diameter and 18 inches in length. Both rolls of the latter machine are power-driven and operate at a differential speed giving a rubbing effect.

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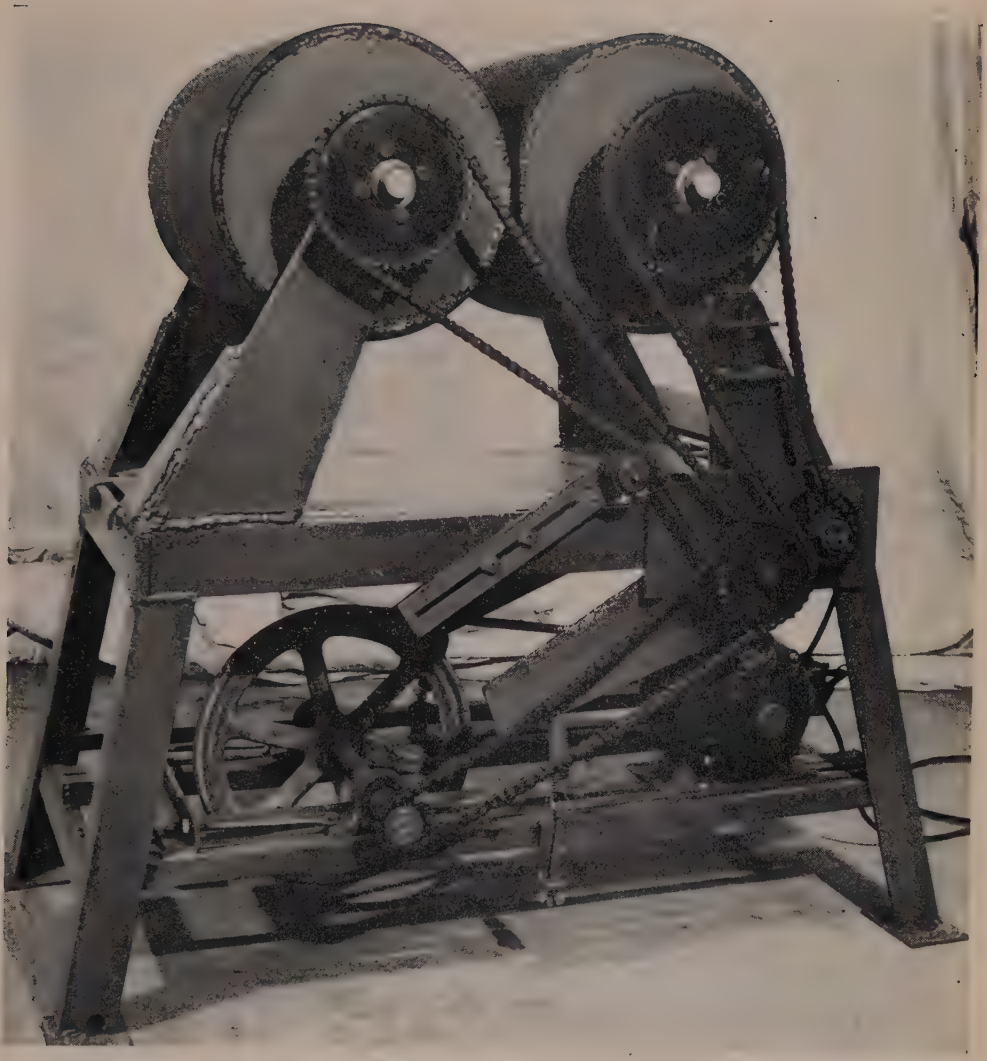

FIGURE 1. Photograph showing general view of thresher.

FIGURE 2. Photograph showing operation of thresher.

One roll of the pair rotates at 50 r.p.m. and the other at 68 r.p.m. This latter model has proven satisfactory for threshing a wide range of crops including wheat, oats, barley, flax, soybeans, radishes, onions, alfalfa and rapeseed.

OPERATION

A cotton or paper bag is placed over the head end of each sheaf and tied in place at the time of harvest. When the material is thoroughly dry, the head end of the sheaf (enclosed in the bag) is passed through the rolls several times (as shown in Figure 2) until the seed is threshed clean from the straw. The straw is then pulled out of the bag and discarded. The grain is cleaned in a Bates Aspirator (1) or a conventional seed cleaner.

DISCUSSION

The advantages of the roller thresher compared to the conventional cylinder machine are:

(1) Mechanical damage to the seed is reduced significantly. In tests made on the 1953 and 1954 crops, one-half of a wheat rod-row plot was threshed in a Kemp cylinder thresher and the remaining portion was threshed in the roller machine. Seed obtained from the cylinder thresher had 64.5 per cent damaged kernels compared to 6 per cent damaged kernels in the seed threshed with the roller machine. Further tests confimed this advantage. Similar trials with flax gave 44 per cent and 4.5 per cent damaged kernels, respectively. Threshing damage to softer seeded crops may be reduced even more significantly.

(2) Mixing of seed in the threshing operation is completely eliminated.

(3) It is not necessary to clean the machine between varieties.

(4) There is no loss of seed during the threshing operation. Small or light kernels are retained in the bag giving a closer approximation to the true yield.

(5) No adjustments are necessary for threshing different kinds of crops.

(6) The machine is extremely simple to operate.

(7) Construction costs are relatively low compared with those of cylinder machines now in use.

Arrangements have been made for the manufacture of this machine.

ACKNOWLEDGEMENTS

The authors are indebted to W. E. Clark, Photographer, Laboratory of Plant Pathology, Winnipeg, for the photographs, and to H. A. H. Wallace, Pathologist, Laboratory of Plant Pathology, Winnipeg, for determining the percentage of damaged kernels in the seed from the two threshing methods.

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ON THE ROUTINE DETERMINATION OF CHROMIC OXIDE IN FECES[1]

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[Received for publication January 16, 1956]

ABSTRACT

A modified photometric procedure is described for the determination of Cr_2O_3 in feces. Ashed material is fused in a furnace with a flux of potassium nitrate, sodium carbonate and sodium hydroxide, and the absorbancy of the dissolved fused material is read at 400 mμ instead of 375. The range of concentrations that can be measured without dilution adjustments has been increased and there is no destruction of the nickel crucibles.

INTRODUCTION

Chromic oxide (Cr_2O_3) is used to an increasing extent as reference material in pasture research with grazing animals. The photometric determination of chromic oxide in feces has been described by Dansky and Hill (1) and Schürch *et al.* (4). A rapid destruction of the nickel crucibles is a common experience with this method. In an attempt to reduce the cost in both time and equipment the procedure described below has been adopted in this laboratory.

MATERIALS AND METHODS

General Description of Method

A representative sample of dried feces is ashed in a nickel crucible and the ash fused with a flux of potassium nitrate, sodium carbonate and sodium hydroxide. This flux is of the type employed by Edin *et al.* (2) and Kane *et al.* (3) with titrimetric procedures. The fused material is dissolved in water and the absorbancy of the solution determined at 400 mμ in a Beckman Model B Spectrophotometer.

Equipment

Muffle furnace with thermostatic control; nickel crucibles, 75 ml. capacity, 53 mm. diameter at top and 57 mm. height; pieces of nickel wire No. 14 B & S gauge about 10 cm. long having a loop at one end bent to fit the bottom of the crucibles; Beckman Model B Spectrophotometer.

Reagents

A 190 gm. KNO_3 mixed well with 100 gm. Na_2CO_3.
B NaOH pellets.
C Hydrogen peroxide, 30% H_2O_2.

Procedure

Into a nickel crucible weigh a representative sample of dried feces containing 10 to 40 mg. of chromic oxide and ash overnight at 600° C. Add approximately 5.8 gm. of reagent A and 5.6 gm. of reagent B, mix thoroughly, and fuse for two hours at 600° C. These two reagents may be added with a calibrated glass scoopula to avoid weighing. Remove the

[1] Contribution No. 304, Chemistry Division, Science Service.

crucibles from the furnace while the fused mixture is still hot and insert a nickel wire loop into each crucible. When the cake has formed after cooling, grasp the wire and loosen the cake by applying a flame to the crucible, and place both cake and crucible in a 400-ml. beaker. Cool and add enough water to submerge both crucible and cake. Dissolution is rapid if the hard cake is suspended in the water from the side of the beaker using the nickel wire as a hook. When dissolution is complete, rinse each crucible and add all washings to the contents of the appropriate beaker. Add approximately 1 ml. of hydrogen peroxide and allow the solution to stand for 3 hours. Filter through Whatman No. 42 filter paper on a Büchner funnel and wash thoroughly. Transfer the filtrate into a 1000-ml. volumetric flask and make up to volume with distilled water. Measure the absorbancy at 400 mμ in the spectrophotometer against a distilled water blank and convert the absorbancy readings to mg. of chromic oxide by employing the equation of a standard curve established with known amounts of chromic oxide.

RESULTS AND DISCUSSION

A standard curve was established with 15 individual determinations of amounts of chromic oxide ranging between 10 and 40 mg. When the instrument was calibrated with the mercury vapour lamp at 404.7 mμ, the equation of this curve (Figure 1) was $x = 43.46\ y$, where x = mg. Cr_2O_3 in fecal sample and y = absorbancy of the solution (1 litre) at 400 mμ.

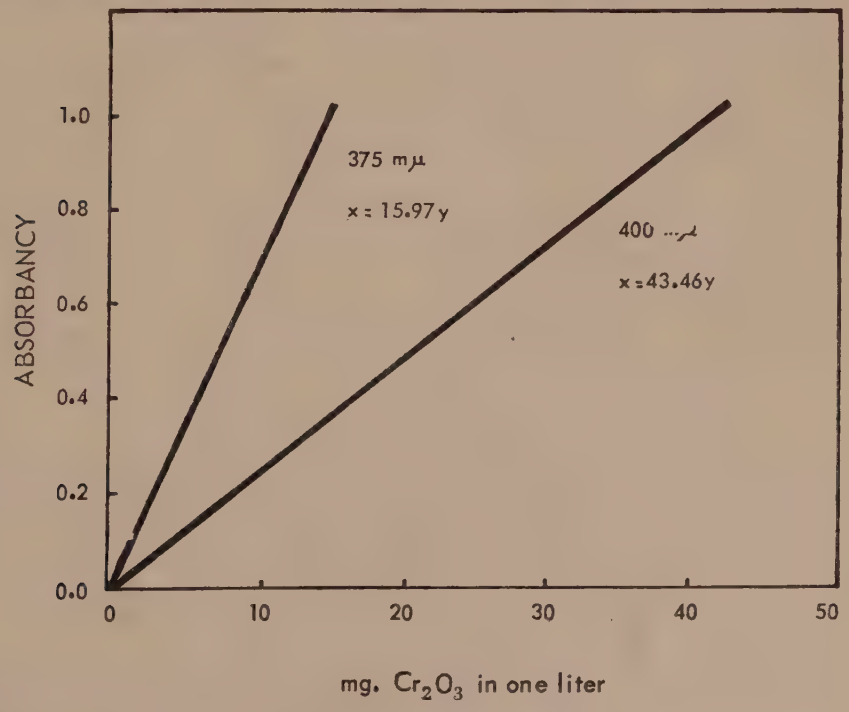

FIGURE 1. Calibration curves at two different wavelengths.

TABLE 1.—COMPARISON BETWEEN TWO METHODS OF DETERMINING FECAL CHROMIC OXIDE

Sample No.	Na_2O_2 method mg.	KNO_3 method mg.	Difference between methods
1	26.73	26.25	− 0.48
2	24.78	24.78	0.00
3	24.99	25.65	+ 0.66
4	24.78	25.43	+ 0.65
5	25.21	25.21	0.00
			Average: + 0.16

A standard curve was also established at 475 mμ which, according to Dansky and Hill (1), corresponds to a maximum of absorption. This curve, $x = 15.97\ y$, is shown in Figure 1. It can be seen that at this wavelength a maximum concentration of only 12 mg. could be determined without extra dilutions. Although the method is more sensitive at 375 mμ, it is more convenient at 400 mμ; without losing appreciable sensitivity a wider range of concentration with sufficiently large fecal samples may be determined without dilution adjustments. Hence the 400 mμ wavelength was adopted in this laboratory.

Calculation of fiducial limits (5) indicates that 96 per cent of the determinations in duplicate will be within $\pm$ 0.85 per cent of the true value.

A comparison between this method and the Na_2O_2 method described by Schürch *et al.* (4) is shown in Table 1. No significant difference was found between the two methods.

This procedure is simple and efficient. A competent operator can carry out twenty determinations in a $7\frac{1}{2}$-hour day when the routine procedure is well established.

The destruction of the nickel crucibles has been virtually eliminated by this procedure. Some crucibles which have been used more than twenty times are still in excellent condition.

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INFLUENCE OF ADDITIONS OF LIME TO SOILS ON THE AVAILABILITY OF POTASSIUM AND OTHER CATIONS FOR ALFALFA[1]

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[Received for publication January 4, 1956]

ABSTRACT

In a greenhouse experiment comprising six soils, varying in reaction from about pH 5.5 to 6.0, addition of calcium hydroxide had no appreciable effect on exchangeable potassium but resulted in a slight reduction in water-soluble potassium in some of the soils. With successive increases in the pH of the soils and associated increases in exchangeable and water-soluble calcium, there was an appreciable reduction in exchangeable magnesium.

The potassium and magnesium contents of alfalfa grown on the soils tended to decrease with liming, whereas the calcium content increased. Increase in yield resulting from addition of potassium fertilizer was usually accompanied by a decrease in the sum of cations in the plants.

Cropping resulted in a marked reduction in the exchangeable potassium. The decline in exchangeable potassium in the clay soils accounted for a smaller proportion of the potassium removed by the crop than did the decline in the sandy soils.

INTRODUCTION

In eastern Ontario, where the soils vary in reaction from medium acidity to slight alkalinity, there has been some uncertainty with respect to the advisability of liming the slightly acid soils before growing alfalfa. In the fall of 1951, studies were begun to ascertain the effect of soil reaction on the availability of phosphorus and potassium for alfalfa grown in greenhouse tests. The availability of phosphorus as influenced by liming was discussed in a previous paper (4). The present paper is concerned with the effects of liming on the availability of potassium. In addition, consideration is given to the amounts of calcium and magnesium in the soils and plants in relation to the lime treatments.

The literature on the influence of liming on the potassium status of soils and on the absorption of potassium by plants has been summarized by Peech and Bradfield (7) and Pierre and Bower (8). More recently, York, Bradfield and Peech (11) reported that additions of lime up to 78 per cent base saturation resulted in a reduction of both water-soluble and exchangeable potassium in Mardin silt loam. In the presence of excess $CaCO_3$, the exchangeable potassium was further reduced, but the concentration of potassium in a water extract was increased over that obtained for 78 per cent saturation. York, Bradfield and Peech (12) found that absorption of potassium in alfalfa was not influenced appreciably by other cations but that added potassium greatly reduced absorption of calcium and magnesium.

[1] Joint contribution from the Division of Field Husbandry, Soils and Agricultural Engineering, Experimental Farms Service, and the Chemistry Division, Science Service. (Contribution No. 302, Chemistry Division). Taken from a part of a thesis submitted by the author in partial fulfilment of the requirements for the Ph.D. degree, Michigan State College.

The yield of alfalfa grown with and without applied potassium, the cation contents of the crop, and the amounts of exchangeable and water-soluble cations extracted from the soils were used to evaluate the effects of liming in the present investigation.

MATERIALS AND PROCEDURE

Ten pounds of air-dried surface samples of six soils were placed in glazed one-gallon pots. The amounts of lime required to raise the pH of the soils were determined by the method of Dunn (2). Calcium hydroxide was mixed throughout the soils to provide different pH levels up to slightly above the neutral point. Soil moisture was adjusted by surface applications of water. After a period of one month, fertilizer treatments were applied to the soils at each pH level. The treatments relevant to this discussion were (1) no potassium; and (2) KCl at the rate of 200 lb. of K_2O per acre. In both series, equal amounts of calcium dihydrogen phosphate and gypsum (simulating superphosphate) were applied at the rate of 200 lb. of P_2O_5 per acre. The fertilizer and lime treatments were randomized and replicated four times. Alfalfa was seeded and later thinned to 10 plants per pot. The yield data represent the total air-dry weight of the crop obtained from four cuttings. Further detail with respect to the soils and greenhouse technique was presented in the previous paper (4).

Composite samples of the plant material from all cuttings and replications of each treatment were prepared for analysis. The plant samples were ashed by wet digestion with sulphuric, nitric and perchloric acids as described by Piper (9). Calcium and potassium contents of the ash were determined by the micro-methods of Peech, Alexander, Dean and Reed (6). Magnesium was precipitated as magnesium ammonium phosphate and determined from the phosphorus content of the precipitate using the method of King (3).

Soil samples were air-dried and passed through a 2-mm. screen. The pH was determined by means of a glass electrode using a 1 : 2.5 soil : water ratio. Exchange capacity and exchangeable bases were determined by the methods of Peech, Alexander, Dean and Reed (6), the micro-methods being used for the bases. Exchangeable hydrogen was determined by the method of Schollenberger and Simon (10). The procedure for water-soluble potassium was similar to that employed for exchangeable potassium except that water was used instead of ammonium acetate. Water-soluble calcium and magnesium were extracted by shaking a 1 : 4 soil : water suspension for a 3-day period. Calcium was separated as the oxalate and determined by titration with potassium permanganate. After separation of the calcium, magnesium was determined by titration with versene as described by Cheng and Bray (1).

RESULTS AND DISCUSSION

Effect of Lime on the Cation Content of Soils

The pH and exchangeable cation properties of the soils following incubation with different rates of calcium hydroxide, but before seeding alfalfa, are shown in Table 1. Except for some of the samples limed to the higher pH levels, the sum of the exchangeable cations in each soil agreed reason-

Table 1.—Exchangeable cation content of soils limed to different pH levels

(Means of two determinations)

Rate of $Ca(OH)_2$/acre	Before potting	Before seeding but after incubation of soil in pots						Base saturation[1]
	Exchange capacity/100 gm.	pH	Exchangeable cations/100 gm.					
			Ca	Mg	K	H	Total	
lb.	m.e.		m.e.	m.e.	m.e.	m.e.	m.e.	%
			Manotick loamy sand					
0	8.46	5.6	2.46	0.37	0.12	5.56	8.51	34
1800	—	6.1	4.01	0.29	0.11	3.69	8.10	56
3800	—	6.6	6.15	0.27	0.11	2.55	9.08	70
5800	—	7.0	7.64	0.24	0.11	1.23	9.22	86
8200	—	7.5	10.31	0.22	0.11	0.00	10.64	100
			Marionville silt loam					
0	17.00	5.4	6.53	3.93	0.24	6.83	17.53	60
1600	—	5.9	7.94	3.85	0.24	4.65	16.68	73
3200	—	6.5	9.73	3.80	0.22	3.30	17.05	81
5800	—	7.0	13.65	3.39	0.22	1.63	18.89	90
8200	—	7.3	15.36	3.04	0.22	0.00	18.62	100
			Bearbrook clay loam					
0	16.11	5.3	5.39	1.75	0.30	7.50	14.94	53
2400	—	5.9	8.25	1.82	0.29	5.06	15.42	69
4000	—	6.4	10.41	1.78	0.30	3.55	16.04	78
6800	—	7.0	13.60	1.44	0.29	1.71	17.04	89
9600	—	7.4	17.24	0.88	0.29	0.00	18.41	100
			St. Thomas fine sandy loam					
0	10.33	5.8	3.02	0.45	0.13	5.60	9.20	46
2800	—	6.5	5.80	0.43	0.11	3.06	9.40	70
5800	—	7.1	8.63	0.40	0.11	1.06	10.20	90
8800	—	7.5	11.99	0.38	0.11	0.00	12.48	100
			Mountain sandy loam					
0	12.57	5.8	6.33	1.75	0.29	4.55	12.92	64
1400	—	6.3	7.39	1.60	0.28	2.99	12.26	76
3800	—	7.0	10.19	1.42	0.27	1.14	13.02	91
6200	—	7.6	12.33	1.12	0.30	0.00	13.75	100
			Rideau clay					
0	17.89	5.9	9.52	4.23	0.56	4.49	18.80	75
800	—	6.2	10.31	4.09	0.56	2.95	17.91	84
2400	—	6.8	12.15	4.02	0.56	2.22	18.95	88
4600	—	7.4	14.76	3.63	0.56	0.00	18.95	100

[1] Calculated from values for exchange capacity and exchangeable hydrogen.

ably well with the corresponding value obtained for exchange capacity. The samples limed to about the neutral point were approximately 90 per cent base saturated. For similar pH levels in the more acid range, the per cent base saturation values for the sandy soils were lower than those found for the clay soils.

Application of lime had no pronounced effect on the exchangeable potassium content of the soils. In most instances, however, the values for exchangeable potassium in the limed samples of all soils except Rideau were slightly lower than the corresponding values for the unlimed samples. With increasing rates of lime and the associated increase in exchangeable calcium, there was an appreciable decrease in the amount of exchangeable magnesium in each of the soils. These results are in agreement with those of Meyer and Volk (5) who reported that calcitic materials exercised a repressive effect on the exchangeable magnesium.

TABLE 2.—WATER-SOLUBLE CATION CONTENT OF SOILS LIMED TO DIFFERENT pH LEVELS

(Single values for Ca, means of 2 for Mg and of 4 for K per 100 grams of soil)

pH	Ca	Mg	K	pH	Ca	Mg	K
	m.e.	m.e.	m.e.		m.e.	m.e.	m.e.
	Manotick loamy sand				*Marionville silt loam*		
5.6	0.19	0.08	0.026	5.4	0.18	0.14	0.018
6.1	0.36	0.08	0.019	5.9	0.29	0.18	0.017
6.6	0.49	0.08	0.021	6.5	0.40	0.19	0.017
7.0	0.70	0.07	0.019	7.0	0.57	0.21	0.015
7.5	0.99	0.05	0.022	7.3	0.91	0.19	0.013
	Bearbrook clay loam				*St. Thomas fine sandy loam*		
5.3	0.13	0.09	0.026	5.8	0.27	0.11	0.028
5.9	0.32	0.11	0.020	6.5	0.44	0.10	0.021
6.4	0.50	0.12	0.023	7.1	0.65	0.10	0.020
7.0	0.73	0.11	0.019	7.5	0.93	0.08	0.020
7.4	1.44	0.08	0.021				
	Mountain sandy loam				*Rideau clay*		
5.8	0.30	0.17	0.038	5.9	0.34	0.23	0.039
6.3	0.27	0.15	0.035	6.2	0.44	0.22	0.041
7.0	0.72	0.15	0.035	6.8	0.51	0.23	0.037
7.6	0.98	0.13	0.037	7.4	0.83	0.20	0.037

The data in Table 2 indicate that liming tended to result in a slight decrease in water-soluble potassium. Although the differences between the values for the limed and unlimed samples were small, nevertheless the amounts of water-soluble potassium in 20 of the 21 limed samples were lower than those found for the corresponding unlimed samples. Water-soluble magnesium in four of the soils tended to decrease with liming, particularly where the pH was raised above the neutral point. The results for the Marionville and Bearbrook soils indicate a slight increase in the amounts of water-soluble magnesium with addition of lime at the lower rates but this increase was followed by a decline in the values at the higher rates. Application of lime increased the water-soluble calcium, the relative increase being greater than that shown for exchangeable calcium.

Yield and Cation Content of Alfalfa

The yield and calcium, magnesium and potassium contents of alfalfa grown with and without applied potassium in soils previously limed to different pH levels are presented in Table 3. In most instances, significant increases in the yield of alfalfa occurred as a result of raising the pH level above that of the unlimed soil. Varying the soil reaction from about 6.5 to above the neutral point, however, resulted in no appreciable differences in yield except on Mountain sandy loam. As reported in the previous paper (4), the yield of alfalfa grown without phosphorus fertilizer tended to increase with increasing pH levels to above the neutral point, and there was evidence that the increase in yield was associated with greater availability of phosphorus in the soils. Application of potassium increased the yield of alfalfa significantly, except on the limed samples of Rideau clay and the samples of Bearbrook clay loam limed to or above the neutral point. The effect of applied potassium on yield was more pronounced on the limed than on the unlimed samples of Manotick loamy sand.

Table 3.—Influence of soil reaction on the yield and cation content of alfalfa grown in the greenhouse

pH of soil	Yield of alfalfa per gallon pot[1]		Cation content of alfalfa per 100 gm.[2]					
			No K added			K added		
	No K added	K added	Ca	Mg	K	Ca	Mg	K
	gm.	gm.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
Manotick loamy sand								
5.6	28.9	38.0	130	40	18	110	30	35
6.1	29.6	45.6	140	34	12	116	24	24
6.6	30.0	47.7	142	34	12	125	23	26
7.0	31.6	51.0	140	31	11	136	22	28
7.5	32.1	49.2	154	29	9	139	23	28
	L.S.D. (0.05) = 4.0							
Marionville silt loam								
5.4	35.4	47.8	84	57	24	82	50	37
5.9	40.6	50.4	99	59	13	89	48	31
6.5	44.4	55.1	115	55	14	96	43	28
7.0	46.8	59.3	122	55	14	107	43	26
7.3	44.1	60.5	133	55	17	109	42	24
	L.S.D. (0.05) = 6.7							
Bearbrook clay loam								
5.3	48.1	58.4	83	44	35	81	36	46
5.9	66.3	72.6	111	41	27	88	34	40
6.4	70.3	76.9	115	36	23	85	32	33
7.0	75.1	75.1	112	34	25	100	32	32
7.4	75.9	73.9	112	31	25	100	27	29
	L.S.D. (0.05) = 6.4							
St. Thomas fine sandy loam								
5.8	15.1	22.4	149	40	24	122	36	46
6.5	20.1	28.1	162	36	16	145	26	38
7.1	20.3	31.2	177	34	14	151	24	33
7.5	21.2	29.8	184	32	12	167	21	33
	L.S.D. (0.05) = 2.8							
Mountain sandy loam								
5.8	34.5	48.4	86	39	45	83	33	52
6.3	48.0	61.0	91	36	37	83	33	46
7.0	55.5	71.2	101	34	28	100	35	36
7.6	63.1	74.1	114	35	29	102	31	36
	L.S.D. (0.05) = 6.7							
Rideau clay								
5.9	66.6	78.7	74	30	72	65	27	69
6.2	75.4	81.9	60	29	70	57	26	70
6.8	75.9	80.3	75	31	68	74	29	66
7.4	82.6	87.9	79	29	63	79	27	70
	L.S.D. (0.05) = 10.3							

[1] Mean air-dry weight for 4 replications.

[2] Mean of duplicate determinations on composite samples expressed on oven-dry basis.

Associated with the increase in yield of alfalfa resulting from liming, there was a decrease in the potassium content of the plants. Liming tended to decrease the magnesium content of alfalfa. The calcium content tended to increase with increasing pH levels. Addition of potassium increased the potassium content of the plants on all soils except Rideau clay, but reduced the calcium and magnesium contents. Since application of potassium increased the yields on most of the soils, it is not possible to assess the direct effect of added potassium on the absorption of other cations.

The relative variation in the amounts of the different cations in the plants in relation to soil reaction with and without added potassium is shown by the data in Table 4. In most instances, the Ca:Mg: and Ca:K ratios increased with increasing pH levels. There was a trend for slightly higher Mg:K ratios to occur for alfalfa grown on the limed samples than for the crop on the unlimed samples, particularly where no potassium was added.

TABLE 4.—RATIOS AND TOTAL EQUIVALENTS OF CATIONS IN ALFALFA GROWN WITH LIMING AND FERTILIZER TREATMENTS

pH of soil	Cation equivalent ratios						Sum of cations (Ca + Mg + K)	
	No K added			K added				
	Ca/Mg	Ca/K	Mg/K	Ca/Mg	Ca/K	Mg/K	No K added	K added
							m.e./100 gm.	
			Manotick loamy sand					
5.6	3.3	7.2	2.2	3.7	3.1	0.9	188	175
6.1	4.1	11.7	2.8	4.8	4.8	1.0	186	164
6.6	4.2	11.8	2.8	5.4	4.8	0.9	188	174
7.0	4.5	12.7	2.8	6.2	4.9	0.8	182	186
7.5	5.3	17.1	3.2	6.0	5.0	0.8	192	190
			Marionville silt loam					
5.4	1.5	3.5	2.4	1.6	2.2	1.4	165	169
5.9	1.7	7.6	4.5	1.9	2.9	1.6	171	168
6.5	2.1	8.2	3.9	2.2	3.4	1.5	184	167
7.0	2.2	8.7	3.9	2.5	4.1	1.7	191	176
7.3	2.4	7.8	3.2	2.6	4.5	1.8	205	175
			Bearbrook clay loam					
5.3	1.9	2.4	1.3	2.3	1.8	0.8	162	163
5.9	2.7	4.1	1.5	2.6	2.2	0.9	179	162
6.4	3.2	5.0	1.6	2.7	2.6	1.0	174	150
7.0	3.3	4.5	1.4	3.1	3.1	1.0	171	164
7.4	3.6	4.5	1.2	3.7	3.5	0.9	168	156
			St. Thomas fine sandy loam					
5.8	3.7	6.2	1.7	3.4	2.7	0.8	213	204
6.5	4.5	10.1	2.3	5.6	3.8	0.7	214	209
7.1	5.2	12.6	2.4	6.3	4.6	0.7	225	208
7.5	5.8	15.3	2.7	8.0	5.1	0.6	228	221
			Mountain sandy loam					
5.8	2.2	1.9	0.9	2.5	1.6	0.6	170	168
6.3	2.5	2.5	1.0	2.5	1.8	0.7	164	162
7.0	3.0	3.6	1.2	2.9	2.9	1.0	163	171
7.6	3.3	3.9	1.2	3.3	2.8	0.9	178	169
			Rideau clay					
5.9	2.5	1.0	0.4	2.4	0.9	0.4	176	161
6.2	2.1	0.9	0.4	2.2	0.8	0.4	159	153
6.8	2.4	1.1	0.5	2.6	1.1	0.4	174	169
7.4	2.7	1.3	0.5	2.9	1.1	0.4	171	176

Thus, there was some evidence that potassium was decreased slightly more than magnesium as a result of liming and the associated increase in yield. Application of potassium reduced the Ca:K and Mg:K ratios but had no appreciable effect on the Ca:Mg ratio. The sum of the cations in the alfalfa grown on the Marionville and St. Thomas soils tended to increase with increasing pH levels. The highest values for sum of cations were obtained for the crop grown on the St. Thomas fine sandy loam. This soil produced lower yields than the other soils. In many instances, with addition of potassium and increase in yield, there was a decrease in the sum of the cations in the plants.

Allowing for the influence of increased yields, the effect of lime on the cation content of the plants was not greatly at variance with the results for the cations extracted from the soils, except that exchangeable magnesium was reduced considerably with liming. York, Bradfield and Peech (12) found that absorption of magnesium by alfalfa was reduced as the rate of liming was increased. In the present investigation, the effect of liming on the magnesium content of alfalfa was much less pronounced.

Effect of Cropping on Exchangeable Potassium in Soils

The data in Table 5 show that cropping resulted in a considerable reduction in the amounts of exchangeable potassium in the soils. Exchangeable potassium in Manotick loamy sand and St. Thomas fine sandy loam was reduced to a relatively low level. Thus, if there is any tendency for exchangeable potassium to be reduced to a constant level, it appears that such a level may be very low in some soils. The values for decrease in

Table 5.—Effect of cropping on the amounts of exchangeable potassium in soils

(Exchangeable K values are means of two determinations; based on 100 grams of soil)

pH of soil	Exchangeable K in soil			pH of soil	Exchangeable K in soil		
	After harvest	Decrease from cropping	Decrease as per cent of K removed by crop[1]		After harvest	Decrease from cropping	Decrease as per cent of K removed by crop[1]
	m.e.	m.e.			m.e.	m.e.	
	Manotick loamy sand				*Marionville silt loam*		
5.6	0.04	0.08	76	5.4	0.17	0.07	41
6.1	0.05	0.06	87	5.9	0.17	0.07	65
6.6	0.04	0.07	100	6.5	0.17	0.05	42
7.0	0.05	0.06	85	7.0	0.16	0.06	46
7.5	0.04	0.07	117	7.3	0.15	0.07	48
	Bearbrook clay loam				*St. Thomas fine sandy loam*		
5.3	0.16	0.14	41	5.8	0.05	0.08	111
5.9	0.17	0.12	33	6.5	0.05	0.06	93
6.4	0.18	0.12	36	7.1	0.04	0.07	125
7.0	0.17	0.12	32	7.5	0.02	0.09	177
7.4	0.15	0.14	37				
	Mountain sandy loam				*Rideau clay*		
5.8	0.12	0.17	55	5.9	0.36	0.20	21
6.3	0.12	0.16	45	6.2	0.35	0.21	20
7.0	0.10	0.17	55	6.8	0.39	0.17	16
7.6	0.08	0.22	60	7.4	0.35	0.21	20

[1] $\frac{\text{(Exchangeable K before seeding—exchangeable K after harvest)} \times 100}{\text{Uptake of K by top growth of alfalfa grown without K fertilizer}}$

exchangeable potassium, expressed as a percentage of the uptake of potassium by the harvested crop, show that the decline in exchangeable potassium in the clay soils accounted for a smaller proportion of the potassium removed by the crop than did the decline in the sandy soils. Since the amounts of potassium in the roots were not considered, the values for decline in soil potassium in relation to uptake were excessive and for two of the soils exceeded 100 per cent.

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SEASONAL YIELD AND NITROGEN CONTENT OF THREE GRASSES GROWN SINGLY AND IN MIXTURES[1]

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[Received for publication January 24, 1956]

ABSTRACT

To test the assumption that each grass species will maintain its individual seasonal-growth trend when grown in a mixture of species, plots were established in 1952 on irrigated land at the Experimental Farm, Lethbridge, Alberta. Smooth brome grass, orchard grass, and creeping red fescue were grown alone, and in all combinations with each other and with white Dutch clover. Seasonal growth trends and yields of each species, alone and in associations, were compared for five clipping harvests in 1953 and 1954. Total yields of mixtures were also compared, and the contribution of each species was considered. Per cent protein of the separated species was determined in 1953.

Competition between species in the mixtures prevented the weaker competitors from exhibiting their individual characteristics. Orchard grass was the strongest competitor, bromegrass was next, and creeping red fescue was the weakest. White Dutch clover was almost completely dominated by orchard grass, and affected to a lesser degree by the other two grasses.

The inclusion of white Dutch clover in the mixture increased protein content of the grasses, and helped to maintain yields over the season in proportion to its amount in the sward.

INTRODUCTION

Throughout the grazing season animal herbage requirements remain relatively constant while herbage production from pastures usually is variable. Several practices have been employed in an attempt to obtain more uniform seasonal production and overcome periods of feed surplus or feed shortage. One practice has been to seed pasture mixtures comprised of several species. This is based on the observation made by many workers, including Sinclair (2), who stated in 1885—"From the spring until the end of autumn there is not a month but it is the season of luxuriance of one or more grasses." Woodman *et al.* (16) applied this observation to cultivated pastures, and stated that a requirement for a good pasture was—"many species in correct proportion with different periods of growth".

Further, it has been suggested by authorities such as Braun-Blanquet (4) that aerial and subterranean layering makes possible the co-existence of several differently adapted species, and permits maximum utilization of an area. There is evidence such as that of Burton (5) who noted that several species of southern grasses, belonging to the same genus, had root systems concentrated at different depths in the soil.

In practice, complex mixtures generally have not provided uniform, season-long pasture. Most mixtures follow similar patterns of production, determined by factors other than species composition. This brings up the question of whether it is correct to assume that each species maintains its individual characteristics in a mixture.

[1] Contribution from the Forage Crops Division, Experimental Farms Service. This paper is based partly on a thesis presented by the senior author to the Graduate School, Utah State Agricultural College, in partial fulfilment of the requirements for the degree of Master of Science in Agronomy.

Henson and Hein (9) found that Kentucky bluegrass dominated eight different pasture mixtures after two years. The complex mixtures appeared to yield a little more in the first two years, but seasonal variations were not diminished. Williams (15) and Davies *et al.* (7) noted that orchard grass soon dominated alfalfa grown in association with it. Roberts and Olsen (13) and Aberg *et al.* (1) grew grass and legume species in association in different combinations, and noted that an increase in yield of one species usually resulted in a decrease in yield of another. Comstock and Law (6) found that a mixture of orchard grass, bromegrass, and alfalfa, clipped as pasture, did not yield more than bromegrass and alfalfa, or orchard grass and alfalfa.

It has been observed (8) (11) (14) that defoliation before the grass becomes too tall helps to maintain ladino or white clover in the sward. Such defoliation has also given more uniform seasonal production (14). It is possible the clover is partly responsible for more uniform seasonal production. Mulder (12) observed that legumes not only supply nitrogen for grasses, but also tend to fill in the gap left by the slow growth of grasses in midsummer. However, data of Johnstone-Wallace (10) indicated that, when Kentucky bluegrass and wild white clover were grown in association, there was greater fluctuation in seasonal production than when either was grown alone.

The experiment reported herein was designed to compare total production and seasonal growth trends of three grasses grown singly and in mixtures. The mixtures included each grass grown with clover only, and all possible combinations of two and three grasses with clover. Botanical analyses of the mixtures were made, and nitrogen determinations done on the separated species. The work was done at the Experimental Farm, Lethbridge, Alberta, from 1952 to 1954.

MATERIALS AND METHODS

Species used in the study were orchard grass *Dactylis glomerata* L. (Lethbridge selection), smooth bromegrass *Bromus inermis* Leyss. (northern commercial), creeping red fescue *Festuca rubra* L. (commercial), and white Dutch clover *Trifolium repens* L. (commercial). The grasses were grown in single species cultures, and in all combinations with each other and with white Dutch clover. Following are the mixtures seeded, and rates of seeding in pounds per acre:

Mixture number	*Species and pounds per acre*
1	Brome 15
2	Orchard 10
3	Creeping red fescue 12
4	Brome 15, white Dutch clover 3
5	Orchard 10, white Dutch clover 3
6	C.R. fescue 12, white Dutch clover 3
7	Brome 7.5, orchard 5, W.D. clover 3
8	Brome 7.5, C.R. fescue 6, W.D. clover 3
9	Orchard 5, C.R. fescue 6, W.D. clover 3
10	Brome 5, orchard 3.3, C.R. fescue 4, white Dutch clover 3.

Seeding was done on irrigated land on May 3, 1952. The land was summerfallowed the preceding year, and was in alfalfa for several years before that time. The soil was a sandy loam, with good subsurface drainage. Seeds were drilled in 7-inch rows, to a depth of one-half inch, with a tractor drawn, multiple V-belt seeder. No companion crop was used. Earlier cropping history of the land had included the growing of inoculated clovers, so further inoculation of clover seeds was considered unnecessary. Plots were 9 feet by 28 feet, and replicated five times in a randomized complete block design. There were no borders between the plots.

Plots were irrigated lightly by sprinker to help establish stands, following which irrigations were made two or three days after cutting. Two inches of water were applied at each irrigation. No fertilizer was applied in 1952. In May, 1953, ammonium phosphate, 11-48-0, and ammonium nitrate, 33.5-0-0, were uniformly broadcast over the plot area. The rate was 50 lb. each of N and P_2O_5 per acre. This application was repeated in November, 1953.

Plots were harvested twice in 1952, and five times each in 1953 and 1954. The main objective in 1952 was to determine the proportion of each species in the mixtures soon after seeding. The 1953 harvests were made at approximately 30-day intervals, but weather conditions prevented adherence to a rigid schedule. Harvest dates in 1953 were May 27, June 30, July 30, September 4, and October 5. In 1954, an attempt was made to harvest the plots each time growth reached a height of ten inches. 1954 harvest dates were June 3, June 23, July 14, August 30, and October 6.

Yields were determined by cutting a 3 foot $\times$ 25 foot swath from the centre of each plot and weighing the samples in the field to the nearest ounce. A sample of about 600 grams was taken to the laboratory for dry matter determination. A second sample of about 300 grams of herbage was drawn from each plot for determination of botanical composition. In 1953 these determinations were made by hand separation of species, and in 1954 by visual estimate. The percentages determined for the species were applied to the total plot yield, and the yield of each species calculated.

Data for each species were analysed separately. Each of the grasses was present in five plots of each replicate providing five comparisons for each species. For example, brome was grown (*a*) alone; (*b*) with clover; (*c*) with orchard grass and clover; (*d*) with creeping red fescue and clover, and (*e*) with orchard grass, creeping red fescue and clover. Yield of brome only was considered under these five treatments. Orchard grass and creeping red fescue yields were analysed similarly. White Dutch clover occurred in seven plots of each replicate.

As an aid in interpreting the results of the experiment, the data were summarized using the analysis of variance technique. Because of large differences in yield from Cut 1 to Cut 5, and in the yield of any one species grown in different mixtures, the error variance was heterogeneous. Therefore, actual yields were converted to logarithms for purposes of statistical analysis. The conversion made the error variance more nearly homogeneous.

Orthogonal polynomials were used to isolate linear seasonal trends of each species under different treatments. Variations in these linear effects

were tested for significance by means of the "F" test in the analysis of variance. A significant difference in linear trends meant that the rate of decline of yield from Cut 1 to Cut 5 varied for the different treatments. Total yields of each mixture were analysed similarly. There were ten treatments based on total yields.

Protein (nitrogen × 6.25) determinations using the Kjeldahl method (3) were made on separated species from the botanical samples collected in 1953.

EXPERIMENTAL RESULTS

1953

Inter-species competition resulted in botanical changes in the mixtures as the season advanced. The general effect was that the tall growth of

TABLE 1—PER CENT BOTANICAL COMPOSITION OF PASTURE MIXTURES IN 1953
(Average of 5 replicates)

Mixture No.	Species	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
1	Brome	97	95	93	87	84
	Weeds	3	5	7	13	16
2	Orchard	100	98	94	89	86
	Weeds	0	2	6	11	14
3	Fescue	96	88	76	70	74
	Weeds	4	12	24	30	26
4	Brome	90	81	65	51	42
	Clover	9	17	35	47	56
	Weeds	1	2	0	2	2
5	Orchard	100	96	96	83	66
	Clover	0	3	4	14	32
	Weeds	0	1	0	3	2
6	Fescue	91	57	36	34	35
	Clover	4	37	61	60	60
	Weeds	5	6	3	6	5
7	Brome	20	13	7	19	11
	Orchard	78	83	82	50	43
	Clover	2	4	9	29	44
	Weeds	0	0	2	2	2
8	Brome	91	64	60	47	30
	Fescue	6	11	6	6	6
	Clover	2	22	33	45	62
	Weeds	1	3	1	2	2
9	Orchard	98	92	85	75	35
	Fescue	1	3	2	1	6
	Clover	1	3	12	20	56
	Weeds	0	2	1	4	3
10	Brome	10	12	8	11	10
	Orchard	87	79	80	60	50
	Fescue	1	3	1	2	6
	Clover	1	3	10	25	33
	Weeds	1	3	1	2	1

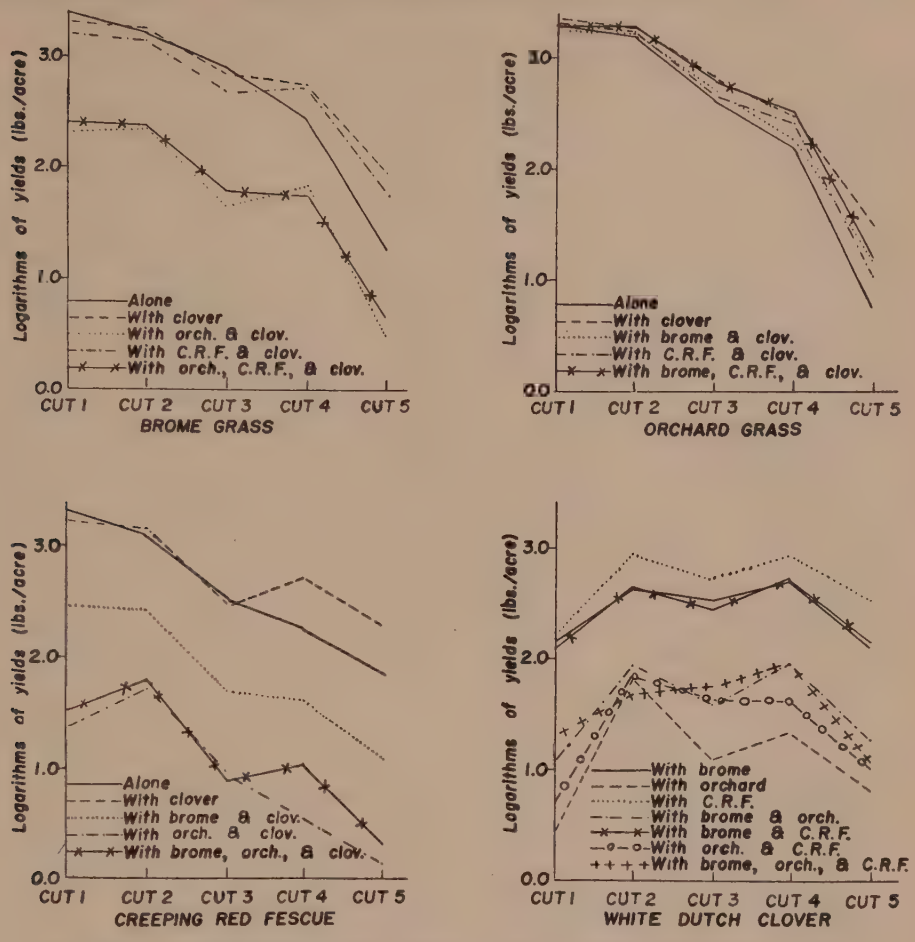

FIGURE 1. Yield trends of three grasses and white Dutch clover grown in different associations of species in 1953.

orchard grass and bromegrass suppressed the low-growing species, creeping red fescue and clover, in the early part of the season. Botanical composition of the mixtures at each cutting is recorded in Table 1. In 1952 the proportion of clover had been low in all mixtures at the time of the first harvest. It had increased considerably by the time of the second harvest in the brome and fescue mixtures, but not in the orchard grass mixtures. Botanical composition at the end of the 1952 season was approximately the same as it was at the second harvest in 1953.

Rapid spring growth resulted in relatively high yields for the first two cuttings, and lower yields from then on. Thus, the general trend was a decline in yield over the season, as measured by the cuttings. Yields of individual species are recorded in Table 2, and seasonal trends are depicted graphically in Figure 1. Since statistical analyses were made with logarithms of the yield data, calculated least significant difference are not applicable to actual yields. Significant differences in yield, at the 5 per cent level and based on the analysis of logarithms of yields, are indicated in the text.

TABLE 2.—YIELD OF GRASSES AND WHITE DUTCH CLOVER IN POUNDS OF DRY MATTER PER ACRE WHEN GROWN IN DIFFERENT ASSOCIATIONS OF SPECIES IN 1953

(Average of 5 replicates)

	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Total
Brome Grass						
Grown with—						
Brome (alone)	2702	1786	871	357	22	5738
Clover	2161	2084	776	667	110	5798
Orchard and Clover	282	291	47	96	5	721
Fescue and Clover	1779	1520	532	588	77	4496
Orchard, Fescue and Clover	358	300	75	74	5	812
Orchard Grass						
Grown with—						
Orchard (alone)	2076	1700	451	179	7	4413
Clover	2402	2149	731	461	41	5784
Brome and Clover	1862	1804	558	359	21	4604
Fescue and Clover	2129	1886	540	278	13	4846
Brome, Fescue and Clover	1909	1890	720	482	35	5036
Creeping Red Fescue						
Grown with—						
Fescue (alone)	2246	1310	355	197	98	4206
Clover	1676	1505	327	540	225	4273
Brome and Clover	327	265	59	49	15	715
Orchard and Clover	26	54	10	4	2	96
Brome, Orchard and Clover	37	68	10	16	3	134
White Dutch Clover						
Grown with—						
Brome	161	430	407	548	146	1692
Orchard	5	71	22	45	18	161
Fescue	182	964	575	946	367	3034
Brome and Orchard	20	72	47	135	15	289
Brome and Fescue	151	542	308	576	153	1730
Orchard and Fescue	10	71	62	73	18	234
Brome, Orchard and Fescue	37	146	70	137	18	408

TABLE 3.—YIELDS OF PASTURE MIXTURES IN POUNDS OF DRY MATTER PER ACRE IN 1953

(Average of 5 replicates)

Mixtures	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Total
1. Brome	2776	1886	921	404	91	6078
2. Orchard	2156	1736	471	199	55	4617
3. Fescue	2324	1481	468	285	254	4812
4. Brome and Clover	2340	2552	1187	1047	388	7714
5. Orchard and Clover	2408	2239	753	517	111	6028
6. Fescue and Clover	1912	2622	932	1583	834	7883
7. Brome, Orchard and Clover	2164	2199	662	606	93	5714
8. Brome, Fescue and Clover	2328	2363	904	1062	372	7229
9. Orchard, Fescue and Clover	2164	2044	617	366	88	5279
10. Brome, Orchard, Fescue and Clover	2360	2420	877	716	132	6505
5 per cent least significant difference						1519

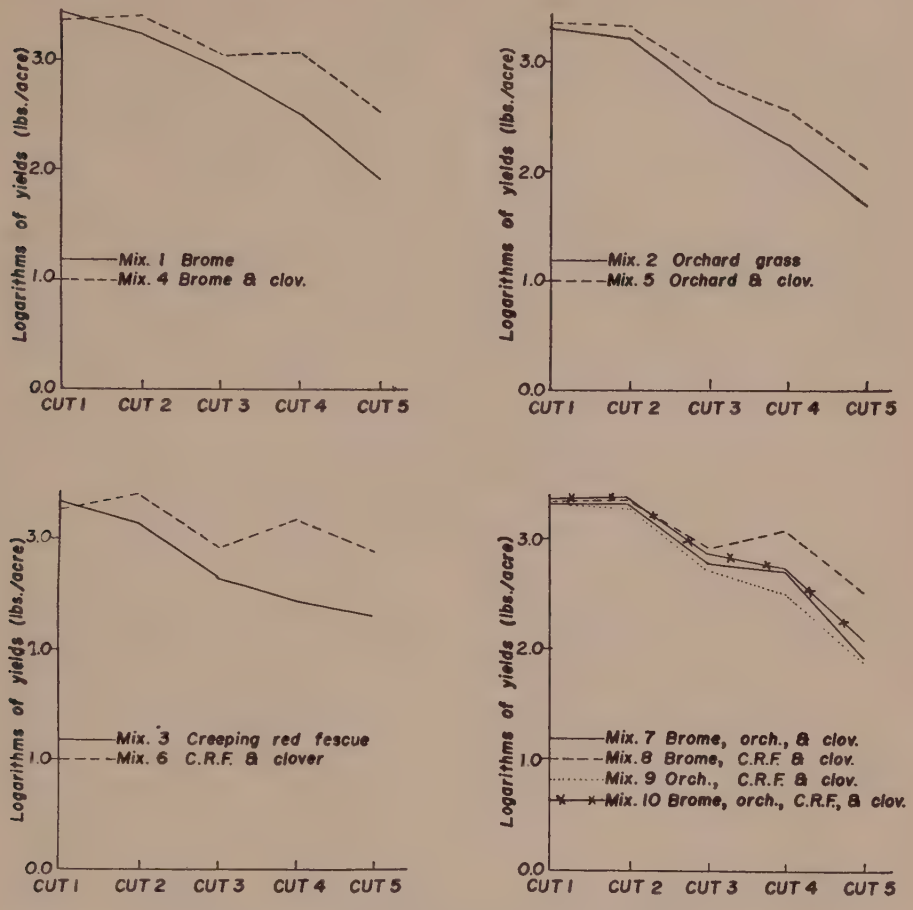

FIGURE 2. Yield trends of pasture mixtures in 1953.

Brome yielded significantly more when grown alone than when in mixtures with orchard grass. Rate of decline of brome yields over the season was slower when it was grown with white Dutch clover, and with fescue-clover, than when grown alone. Orchard grass yields and trends were not influenced through association with the other species. Yield of creeping red fescue was reduced when it was grown with other grasses, but seasonal yield trends were not influenced significantly. When grown with clover only, creeping red fescue yields were not reduced, and rate of seasonal decline was slower. White Dutch clover yielded significantly less in all mixtures containing orchard grass than in non-orchard grass mixtures. Seasonal trends were not affected through association with any of the grasses.

Total yields of mixtures are recorded in Table 3, and trends are depicted graphically in Figure 2. Differences in total seasonal yields of the ten treatments were analysed on the basis of actual yields, since differences were not great. Brome-clover and fescue-clover mixtures yielded more than these grasses when grown alone, but the orchard-clover mixture did not

yield significantly more than orchard grass alone. Mixtures of two or three grasses did not yield more than one grass with clover. Addition of orchard grass to the brome-clover mixture resulted in a decreased yield. Rate of decline in yield over the season was slower for mixtures of one grass and clover, than for the grass grown alone. Incorporation of another grass into the mixture did not reduce the rate of decline further.

Percentage of crude protein was determined for individual species in the first four cuts when sufficient material was available. Unfortunately some samples were too small, and consequently the data for fescue and clover are incomplete. Protein content of the three grasses and clover, in the different associations of species, is recorded in Table 4. For cuts in

TABLE 4.—PER CENT CRUDE PROTEIN IN THREE GRASSES AND WHITE DUTCH CLOVER GROWN IN DIFFERENT ASSOCIATIONS OF SPECIES IN 1953

(Average of 5 replicates)

	Cut 1	Cut 2	Cut 3	Cut 4
Brome Grass				
Grown with—				
Brome	25.8	20.3	17.9	16.6
Clover	25.3	20.7	21.0	19.4
Orchard and Clover	25.9	15.0	18.3	13.7
Fescue and Clover	24.6	19.1	19.6	18.4
Orchard, Fescue and Clover	25.8	15.6	19.1	16.1
	No significant differences			
Orchard Grass				
Grown with—				
Orchard	23.6	10.9	12.6	11.8
Clover	23.2	11.7	12.7	13.5
Brome and Clover	24.5	12.6	14.6	14.4
Fescue and Clover	22.6	12.8	13.9	13.2
Brome, Fescue and Clover	24.4	13.6	15.3	14.2
	No significant differences			
Creeping Red Fescue				
Grown with—				
Fescue	21.7	13.2	12.6	11.0
Clover	22.6	15.4	16.2	15.2
Brome and Clover	21.7	16.7	15.3	14.6
Orchard and Clover	22.0	12.4*	11.2*	12.4
Brome, Orchard, and Clover	22.6	13.8*	13.5*	12.2
5 per cent L.S.D.	N.S.	—	—	2.2
White Dutch Clover				
Grown with—				
Brome	24.7	23.7	22.7	23.5
Orchard	27.4	19.1*	19.7	19.1
Fescue	25.0	22.3	23.0	24.2
Brome and Orchard	27.2	20.3*	19.8	21.1
Brome and Fescue	25.8	23.0*	22.4	22.7
Orchard and Fescue	26.0	20.5*	19.1	20.3
Brome, Orchard, and Fescue	25.4	21.4*	20.3	20.8
5 per cent L.S.D.	N.S.	—	1.7	1.4

* Average has missing values.

TABLE 5.—YIELDS OF PASTURE MIXTURES IN POUNDS OF DRY MATTER PER ACRE IN 1954

(Average of 5 replicates)

Mixture	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Total
1. Brome	1185	543	300	711	56	2795
2. Orchard	1755	374	312	507	38	2986
3. Fescue	970	585	356	840	277	3028
4. Brome and Clover	1674	713	920	1910	218	5435
5. Orchard and Clover	2063	447	544	1020	209	4283
6. Fescue and Clover	1210	983	1112	2072	700	6077
7. Brome, Orchard and Clover	2015	482	644	1435	313	4889
8. Brome, Fescue and Clover	1621	821	1000	1971	353	5766
9. Orchard, Fescue and Clover	1881	447	576	1414	318	4636
10. Brome, Orchard, Fescue and Clover	1881	422	592	1438	249	4582
5 per cent least significant difference						652

which samples were missing, figures are averages of as many replicates as were available.

The protein content of bromegrass and orchard grass, in all associations, was significantly greater in Cut 1 than in the other three cuttings for which determinations were made. However, the different associations had no significant effect on protein content of these grasses. Protein content of creeping red fescue under different treatments could not be analysed statistically for Cuts 2 and 3 because too many samples were missing. However, significant differences were found in Cut 4. Similarly, clover could not be analysed for Cut 2, but there were significant differences in Cuts 3 and 4.

1954

Detailed data for 1954 are not presented, but total yields of mixtures are recorded in Table 5. It was not practical to average data for the two years, because a cutting in 1954 does not necessarily bear a direct relationship to one in 1953.

The general trend of yields over the season, as measured by the cuttings, was a decline for brome, orchard grass, and total yields of mixtures. White Dutch clover yields increased as the season advanced. Creeping red fescue yields increased over the season when grown with other grasses, but decreased when grown alone or with clover only. Orchard grass yields and linear trends were affected by association with clover. The effect was to increase yield of orchard grass, and decrease the rate of decline in yield over the season. Clover was still at a very low level in all orchard grass mixtures, but had increased somewhat from 1953.

DISCUSSION AND CONCLUSIONS

Since 1953 results are reported in the greatest detail most of the discussion will refer to them. Where 1954 results indicate important relationships they will be cited.

The relative competitive ability of the three grass species was established for the conditions of the experiment. Orchard grass was the strongest competitor, bromegrass next, and creeping red fescue the weakest. The effect of competition on yield can be seen in Figure 1. Clover did not compete with the grasses, but rather tended to improve grass growth. The greatest influence on seasonal growth trends of grasses seemed to come from clover. The effect of the grasses on each other was mostly indirect, through limiting clover growth, although an apparent direct effect of one grass on another occurred in the brome-fescue-clover association. There the rate of decline of brome yields over the season was slower than for brome grown alone, but that of fescue yields was not. Therefore, it seems that brome influenced the growth trend of fescue by competing successfully for the available nutrients.

In 1953, the main reason for the rapid decline in growth of the grasses after Cut 2 appeared to be nitrogen deficiency. Several factors lend evidence to this opinion. Orchard grass in particular, and brome to some extent, showed characteristic symptoms of nitrogen deficiency. Protein content of all three grasses fell markedly after Cut 1, despite the fact that herbage was less mature in subsequent cuttings. Clover, which was able to obtain nitrogen from nodule forming bacteria, increased somewhat in yield as the season advanced, and in general maintained a high protein content. Protein content of fescue in Cut 4 was considerably higher when it was grown with clover than when grown alone.

The rate of nitrogen fertilizer application, 50 lb. per acre, had purposely been light in order that differences between clover and non-clover mixtures might be shown. The intense suppression of the clover by orchard grass and bromegrass had not been anticipated.

Undoubtedly lack of available nitrogen was an important factor in limiting growth, but it does not fully explain competition between species. Orchard grass competed successfully with brome, yet when each species was grown alone brome removed more nitrogen from the soil than did orchard grass. From yield and protein data it can be calculated that in 1953 brome grown alone removed approximately 204 lb. of nitrogen per acre, orchard grass 121 lb., and creeping red fescue 118 lb. There is some question as to the competition between grasses and clover being for nitrogen, since clover had an independent source in the root nodules. Orchard grass showed a tendency to affect, adversely, the protein content of grasses growing in association with it. Whether this was due to the competition for nitrogen or for some other element required in protein metabolism is not known.

It seemed possible that in early 1953 lack of light reduced the growth of clover in orchard-clover and brome-clover mixtures. Creeping red fescue never grew higher than about 6 inches, and so did not produce the same shading effect. However, after Cut 2, when the shading effect of brome and orchard grass was not great, clover yields did not increase. Further, in 1954, orchard grass and brome grass were not permitted to grow too tall, yet clover still was held to a very low proportion in the mixture. Thus, although low light intensity may have limited growth at times, it was not the major factor involved.

It appears that many factors may have limited growth and any one species may have been able to compete more successfully for one factor than another. Growth of each species in a mixture would depend, then, on the importance of the factor for which it was unable to compete. Whatever the nature of the competition it did exist, and through it one species was able to influence the growth of another. The effect of clover must be considered beneficial only. It reduced the rate of decline in yield of two of the grasses, and tended to increase the protein content, without reducing the total yield. The influence of the grasses on each other was not beneficial.

Total yields of mixtures (Tables 3 and 5) indicate the value of clover. All mixtures of which clover was a major constituent yielded more, and had more uniform seasonal production, than non-clover or low-clover mixtures. The tables indicate that clover helped to give more uniform production, possibly by two means. First, it may have supplied some nitrogen for the grasses, and second, it filled in the gap left by the slow growth of the grasses. This is in agreement with the statement of Mulder (12), referred to earlier.

Many recommendations for pasture have been based on the premise that the more diverse the characteristics of the component species, the better they will grow in association. Such a premise is not justified on the basis of these results. The species used in this experiment have different types of root systems, different moisture requirements, respond differently to time of season, and grow to different heights. However, these differences could not compensate for limitations in growth factors. The influence of one species on another was such that none could fully express its individual characteristics.

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FRUIT FLIES AND FUNGAL WASTAGE IN PEACHES[1]

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[Received for publication February 7, 1956]

ABSTRACT

In experiments over a 3-year period, it was found that fruit-fly infestation increased both the incidence and the rate of development of brown rot and black mould in harvested peaches when external sources of inoculum were available. In the absence of the flies, the presence of inoculum sources within 2 ft. of baskets of test fruit had no consistent effect on the incidence of wastage. The percentages of brown-rot infections starting at the stem end in the absence and presence of flies were respectively 44 and 60 in the Veteran variety, and 66 and 84 in Elberta. The latter variety is notoriously subject to stem-end injuries at harvest. Sulphur not only gave somewhat better control of brown rot than captan did, but also appeared to have some repellent effect on the flies.

Black mould usually did not appear until the fruit was ripe and was carried very efficiently by the flies from outside sources of inoculum. If black mould inoculum was present on the test fruit when picked, but not in the external source, the fruit flies had little effect on its dissemination. Under these conditions, captan was more effective than sulphur against black mould.

INTRODUCTION

At the height of the peach harvest season it is often necessary to hold the fruit in containers, usually bushel boxes, either in the orchard or in storerooms until the canneries are able to process it. Under such conditions losses from brown rot and other fungi are almost inevitable and often heavy. In the fall of 1952, one enterprising grower followed the development of wastage in peaches in two cannery boxes, one of which was carefully protected from fruit flies by cheesecloth, while the other was left unprotected The experiment was set up on September 28 under trees in the orchard. As the results showed, weather conditions at that time of year were not favourable for brown-rot development. On the twelfth and twenty-eighth days the incidence of rot in the protected box was 7.7 and 32.6 per cent, respectively, while that in the unprotected box was 85.9 and 94.7 per cent. To follow up this exploratory experiment the Entomology Laboratory, Vineland Station, and the Plant Pathology Laboratory, St. Catharines, undertook to investigate the effect of infestations of fruit-flies (*Drosophila* spp.) on the development of brown rot (*Monilinia fructicola* (Wint.) Honey) and black mould (*Rhizopus nigricans* Ehr.) in harvested peaches. The results of three seasons' observations are presented in this paper.

MATERIALS AND METHODS

Rectangular cheesecloth-covered cages, 3 ft. by 3 ft. by 4 ft., were built on wooden bases about a foot deep. Several removable slats were placed on top of each base to form the false floor of the cage proper. The cages were placed in a row on a trestle table set up in an open space outdoors. The lower compartments of some of the cages contained a quantity of

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brown-rotted fruit to serve as an external source of inoculum out of direct contact with the test fruit in the upper compartments. Large mixed cultures of fruit-flies containing *Drosophila melanogaster* Mg., *D. busckii* Coq., *D. repleta* Woll., and *D. immigrans* Sturt. were established on the brown-rotted fruit in some of the cages. The test fruit was picked from several trees in a given block and distributed at random among 18 six-quart baskets, which in turn were divided equally among three cages. The first cage (C), containing only test fruit, was used as a check. The second cage (R) was supplied with the external source of inoculum but no fruit flies and the third (F) with both inoculum and flies. Cages C and R were sprayed with an aerosol containing DDT at the beginning of the experiment and as often as necessary during the experiment to ensure freedom from infestation by the flies. In all experiments, the population of flies in cage F very soon reached the density to be expected when fruit flies are allowed to multiply freely in decaying fruits in a confined space. As the experiments were usually run in late August or in September, conditions were much more favourable both for the ripening and softening of the fruit and for the development of brown rot, than they were in the pilot experiment already described.

In 1953, tests were run on the varieties Oriole and Elberta and seedling selection V-39056, each of which had received the regular sulphur spray program during the growing season. The 1954 and 1955 experiments were carried out with the varieties Veteran and Elberta, each from two blocks of which one was sprayed with captan (*N*-trichloromethylthiotetrahydrophthalimide) and the other with sulphur according to the recommended schedule. Because of the differences in ripening dates the varieties were not tested concurrently. Differences between varieties with respect to brown-rot incidence are therefore not necessarily attributable to differences in varietal susceptibility.

In each experiment, the first record of wastage was taken when brown rot became evident in cage C. The fruit on which records were taken was discarded after examination. Consequently, if a second examination was deemed necessary, three of the six baskets in each cage were left untouched for the second recording. The significance of differences within each variety was determined by an analysis of variance for each complete experiment. For statistical purposes, percentages were transformed to degrees.

RESULTS

In 1953, the incidence of brown rot in any one variety was not significantly affected by the presence or absence of either external sources of inoculum or fruit flies (Table 1). The more rapid rate of brown-rot development in the seedling lot than in the two varieties was undoubtedly due to the higher temperatures prevailing throughout the holding period. Mean temperatures were 65°, 80° and 60° F. during the Oriole, seedling, and Elberta tests, respectively. Showers occurred on the fourth day of the seedling test and on the fifth day of the other two. The outstanding effect of fruit-fly infestation in all three tests was the increase in black mould wastage (Table 1). Furthermore, the incidence of black mould increased in successive tests. These results can be explained by the occurrence of

TABLE 1.—EFFECT OF FRUIT FLIES ON THE PERCENTAGE INCIDENCE OF FUNGAL WASTAGE IN THREE VARIETIES OF PEACHES IN 1953

Type of wastage	Oriole (August 17-24)			Seedling V. 39056 (Aug. 27-Sept. 1)			Elberta (Sept. 21-Oct. 2)		
	C[1]	R	F	C	R	F	C	R	F
	4th day			5th day			8th day		
Incipient B.R.[1]	2.6	3.2	4.3	9.0	8.7	4.4	14.3	6.8	3.9
Advanced B.R.	4.3	6.4	3.4	19.1	22.0	17.7	10.2	6.9	0.0 (?)
Rhizopus sp.	0.0	0.0	0.0	0.0	0.6	*56.6***[2]	0.0	2.9	85.3**[3]
Total	6.9	9.6	7.7	28.1	31.3	*78.7***	24.5	16.6	*89.2***
	7th day						11th day		
Incipient B.R.	7.8	12.8	8.5	Fruit over-ripe all examined on 5th day			11.7	11.6	0.0 (?)
Advanced B.R.	19.4	23.9	18.8				29.1	28.7	0.0 (?)
Rhizopus sp.	1.8	0.0	*37.6***				14.3	0.9	97.1**[3]
Total	29.0	36.7	*64.9***				55.1	41.2	*97.1***

[1] The abbreviations in the tables are those used in the text. *C*—check cage; *R*—cage with inoculum but no flies; *F*—cage containing inoculum and flies; *B.R.*—brown rot.
[2] Differences from check (C) significant at 1 per cent level**.
[3] Brown-rot lesions overrun and obscured by black mould (*R. nigricans*).

black mould in the external sources of inoculum and its build-up there as the season progressed. The flies were obviously responsible for carrying the mould to the test fruit. The black mould in the Elberta check on the eleventh day resulted from the inadvertent use of an old contaminated basket.

In the 1954 experiment with the Veteran variety (Table 2), black mould was of no importance. The effect of fly infestation on brown-rot incidence in the peaches that had been sprayed with captan was relatively slight on the fourth day, but on the seventh was manifested by a pronounced though not significant increase over the check in advanced lesions. A comparison of the total wastage and the relative distribution of incipient and advanced brown rot in the three captan-sprayed lots at both examinations suggests that the flies hastened both the onset and the development of infection. The peaches from the sulphur plot were slower to develop brown rot than those from the captan plot and did not show any increase of infection in the cage that contained flies.

In the Elberta experiment of 1954 (Table 2) wastage was negligible on the seventh day, after which rising temperatures and humidities, accompanied by showers on the ninth day, hastened ripening and favoured brown-rot development. Fly infestation increased the amount of advanced rot in the sulphur-sprayed as well as in the captan-sprayed lot. There was some evidence that black mould had become established in the external sources of inoculum and was being carried over to the test fruit by the flies, particularly on the captan series.

TABLE 2.—EFFECT OF FRUIT FLIES ON THE PERCENTAGE INCIDENCE OF FUNGAL WASTAGE IN VETERAN AND ELBERTA PEACHES IN 1954

Type of wastage	Captan-sprayed			Sulphur-sprayed		
	C	R	F	C	R	
4th day (Sept. 7)						
Experiment with var. Veteran						
Incipient B.R.	3.7	9.5	15.4	11.7	12.7	5.3
Advanced B.R.	11.1	10.5	14.3	5.1	5.7	6.4
Rhizopus sp.	0.0	0.0	0.0	0.0	2.5	0.0
Total	14.8	20.0	29.7	16.8	20.9	11.7
7th day (Sept. 10)						
Experiment with var. Veteran						
Incipient B.R.	16.5	10.4	7.8	6.9	11.5	14.9
Advanced B.R.	22.8	24.7	41.7	26.8	22.5	20.7
Rhizopus sp.	0.0	0.0	0.0	0.0	0.0	0.0
Total	39.3	35.1	49.5	33.7	34.0	35.6
10th day (Oct. 1)						
Experiment with var. Elbert						
Incipient B.R.	27.0	27.3	*17.2*[1]	21.6	18.1	23.6
Advanced B.R.	34.5	38.8	*57.6***	22.6	21.5	*46.0***
Rhizopus sp.	0.0	1.2	*11.1***	0.0	0.0	*5.6**
Total	61.5	67.3	*85.9***	44.2	39.6	*75.2***

[1] Differences from check (C) significant at 5 per cent level*; at 1 per cent level**.

Brown-rot incidence in the Veteran variety followed much the same pattern in 1955 as in 1954, except that the presence of flies affected the number of advanced lesions in the peaches sprayed with sulphur as well as in those sprayed with captan, though to a lesser degree (Table 3). Although temperatures were lower and there were no showers during the 1955 experiment, the general level of brown rot was appreciably higher than in 1954, probably because a record rainfall and high temperatures in August fostered orchard infections and thus tended to raise the inoculum potential on the fruit surface. Pre-harvest conditions along with somewhat higher temperatures during the test period also affected the Elberta experiment, with the result that, by the sixth day, brown rot had reached the same stage of development as that observed on the tenth day in 1954. The flies had much the same effect in both years (Table 3).

TABLE 3.—EFFECT OF FRUIT FLIES ON THE PERCENTAGE INCIDENCE OF FUNGAL WASTAGE IN VETERAN AND ELBERTA PEACHES IN 1955

Type of wastage	Captan-sprayed			Sulphur-sprayed		
	C	R	F	C	R	
6th day (Sept. 7)						
Experiment with var. Veteran						
Incipient B.R.	20.4	17.2	15.2	15.7	12.1	*5.9**
Advanced B.R.	26.5	30.9	*43.4***	19.7	28.3	*35.6**
Rhizopus sp.	2.8	4.9	2.2	9.7	6.1	5.3
Total	49.7	53.0	*60.8***	45.1	46.5	46.8
6th day (Sept. 21)						
Experiment with var. Elberta						
Incipient B.R.	20.3	19.5	18.8	15.9	15.7	7.5
Advanced B.R.	27.9	22.5	*60.9***	23.9	24.5	*65.5***
Rhizopus sp.	5.4	4.5	1.5	13.0	16.9	13.9
Total	53.6	46.5	*81.2***	52.8	57.1	*86.9***

[1] Differences from check (C) significant at 5 per cent level*; at 1 per cent level**.

Black mould caused some wastage in both varieties in 1955, but appeared at random in all cages (Table 3). This random distribution would indicate that some black-mould inoculum was present on the test fruit when it was packed and had not been spread by the flies from the rotting fruit in the bottom compartments of the cages. Once started, black mould spreads very rapidly from fruit to fruit and, when the records were taken, had evidently not been established long enough to be disseminated by the fruit flies. In both varieties, captan was more effective than sulphur against black mould when the inoculum was not introduced from an outside source.

It is possible for fruit flies to affect the incidence of fungal infections either passively, by increasing the spore load over the surface of the fruit during their explorations, or actively, by introducing inoculum into entry sites during feeding or egg-laying. However, fruit flies are considered to be able to attack fruit only through breaks in the skin. The most common injury on peaches severe enough to be vulnerable to the flies is to be found at the stem end, where small flaps of skin are often torn loose or broken off when the fruit is picked. To assess the relative importance of the active and passive roles of fruit flies in spreading inoculum, the percentage of brown-rot lesions originating at the stem end was calculated for each lot in the Veteran experiments of 1955 and in the Elberta experiments of 1954 and 1955. Infections starting at the stem end in the absence and presence of flies were responsible for 44 and 60 per cent, respectively, of the total brown-rot wastage in Veteran and 66 and 84 per cent, respectively, in Elberta.

TABLE 4.—PERCENTAGES OF BROWN ROT LESIONS ORIGINATING AT THE STEM END AND TOTAL PERCENTAGES OF PEACHES WITH BROWN ROT IN THE PRESENCE AND ABSENCE OF FRUIT FLIES IN THE VARIETIES VETERAN (1955) AND ELBERTA (1954 AND 1955)

	Veteran, 1955		Elberta, 1954		Elberta, 1955	
	Stem	Total	Stem	Total	Stem	Total
	Captan-sprayed					
Flies present	33.8	58.6	60.7	74.8	71.1	79.7
Flies absent	19.6	47.5	46.5	63.8	27.8	45.1
Difference	*14.2*	*11.1*	*14.2*	*11.0*	*43.3*	*34.6*
	Sulphur-sprayed					
Flies present	26.1	41.5	54.5	69.6	65.6	73.0
Flies absent	17.7	37.9	22.8	41.9	28.7	40.0
Difference	*8.4*	*3.6*	*31.7*	*27.7*	*36.9*	*33.0*

Note: Coefficient of correlation between increases in stem-end infections and those in total infections in presence of flies = 0.9925 ($P < 0.01$).
In absence of flies, coefficient of correlation between increases in stem-end infections and those in total infections in presence of external sources of inoculum = 0.5044 ($P > 0.1$).

Analysis of variance in the 1955 data showed that differences both within and between varieties were significant at the 0.1 per cent level. From casual observation at the various times, Elberta has long been regarded as more prone than most varieties to stem-end injury. In both Veteran and Elberta, regardless of spray program, the increase in stem-end infections resulting from the activities of the flies was more than enough to account for the accompanying over-all rise in brown-rot wastage (Table 4). When flies were not present, neither the presence nor absence of inoculum had any consistent effect on the relation between stem-end and total incidence of brown rot. Presumably the frequency of stem-end injury varied from basket to basket and from cage to cage.

DISCUSSION

These experiments show that fly infestations almost invariably enhance fungal wastage in peaches held for more than three or four days. Both the degree of enhancement and the type of wastage aggravated by the flies can vary considerably with a number of environmental factors, such as atmospheric conditions, type, density, and location of inoculum, the amount of injury in the fruit, and so on.

One factor of prime importance is the stage of maturity reached during the holding period. Peaches are usually picked at the hard-ripe stage when the skin is firm and when the brown-rot fungus, barring injuries, usually enters the fruit by way of hair sockets (1). As the fruit ripens, the dermal layers first tend to separate from the flesh, that is, the skin starts to "slip", and then they undergo partial disintegration so that, in over-ripe fruit, the skin is very easily broken and offers little resistance to brown rot. It is

scarcely necessary to mention that the rate of ripening varies markedly with temperature. Before the fruit is ripe, atmospheric conditions being the same, incidence of brown rot depends on the spore load, or inoculum potential, on the fruit surface and on the coverage and efficiency of the fungicide used. In the early stages of ripening, the flies appear to play an active part by implanting inoculum in injured areas, and their effect on brown-rot wastage is largely governed by the amount of stem-end injury present. Stem-end injury is chiefly a varietal characteristic, but may be affected by other factors. After the fruit ripens and becomes more susceptible to brown rot, the passive role of the flies in increasing inoculum potential may become more and more important. Black mould appears to be unable to penetrate the defences of the fruit until it is nearly ripe. It was not possible, because of the rapidity of the growth of black mould, to determine the points of entry and their relative importance, but it is probable that the passive role of the flies is at least as important as the active one, as far as the dissemination of this fungus is concerned. In any case, the flies proved to be very efficient carriers of black-mould inoculum.

The conditions that hasten post-harvest ripening are also those that favour brown-rot development, not only by their effect on the fruit itself, but also by their effect on spore germination and mycelial growth. The length of time that peaches can reasonably be expected to be held without refrigeration, before losses from rot begin to make themselves felt, may vary from two or three days early in the season, when mean temperatures may be in the 70's and 80's, up to two or three weeks at the end of the season, when mean temperatures are likely to be in the 50's and low 60's. In either case, heavy infestation by fruit-flies is almost certain to halve the holding period.

In nearly all the tests in which the two fungicides were compared, brown-rot incidence was higher with captan than with sulphur. These differences, however, may have been due less to differences in the effectiveness of the two fungicides than to differences in the maturity of the fruit at harvest. In nearly all the experiments the fruit sprayed with captan appeared to be slightly riper than that sprayed with sulphur. There was not enough evidence available to determine whether maturity was affected by the location of the plots in the orchard or whether captan was absorbed by the peaches and affected their ripening rate. In both Veteran experiments, the increase in brown rot associated with the presence of flies was considerable in the peaches sprayed with captan but negligible in those sprayed with sulphur. These observations suggest that sulphur may be more or less repellent to flies. In Elberta, the repellent effects of the sulphur may account for the lower incidence of black mould in 1955, but they were apparently counteracted by the attraction of the stem-end injuries as far as brown rot was concerned.

Without a thorough clean-up campaign, it is almost impossible to place boxes of fruit in the orchard outside the range of fruit flies frequenting fruit rotting on the orchard floor. Such fruit is usually host to both brown rot and black mould and constitutes a menace to any sound fruit in the orchard, either on the trees or in boxes. A ground spray of DDT would be advisable if fruit is to be held for any length of time in the orchard. Considerable

benefit would also be derived from steam-sterilizing the boxes to minimize fungal contamination each time they are to be used for fruit. A few years ago, a heavy infestation of fruit-flies in an experimental room that contained baskets of peaches was controlled by spraying the walls, floor and ceiling with a heavy dosage of DDT. If this practice is followed in commercial packing sheds or storerooms, spraying of course should be done only when the room is empty to avoid poisonous residues on fruit and containers.

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WHIPTAIL OF CAULIFLOWER IN PRINCE EDWARD ISLAND[1]

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[Received for publication December 28, 1955]

ABSTRACT

Whiptail of cauliflower occurs regularly in most market gardens of Prince Edward Island. Symptoms and effects are described. The disorder is invariably associated with highly acid soils which have received heavy applications of commercial fertilizer. Correction of the deficiency has been achieved by soil or foliar additions of molybdate salts. Applications of 6 to 16 ounces of molybdenum per acre in soil, or 1 to 12 ounces per acre on the foliage gave satisfactory control.

INTRODUCTION

Whiptail of cauliflower is a nutritional disorder caused by a deficiency of molybdenum. It was described first in Long Island in 1924 (1), and since then has been reported and studied in several countries, principally Great Britain and Australia (3, 7). The disease is characteristically associated with acid soils and may be corrected either by liming or by the use of molybdate salts (5).

The purpose of this paper is to record some observations on this disease and on its control in Prince Edward Island.

GENERAL OBSERVATIONS

Whiptail has appeared regularly over the past 5 years in a number of market-gardens in Prince Edward Island where large quantities of commercial fertilizer have been used but where little or no lime or manure has been applied. The disease is characterized by a mild interveinal chlorosis of the leaves of very young plants and later by an incurling of leaf margins, distortion and upright growth of new leaves near the growing point. Leaf blades become ruffled and convoluted and in extreme cases are so inhibited in development that only the bare midribs remain. In serious cases the growing point is completely abortive, leaving only a brown stump. Plants less severely affected may produce loose, ricey curds. In midseason molybdenum-deficient plants are small and upright in habit and commonly exhibit one or more malformed leaves. The defective laminae are abnormally thickened. This condition is apparently due to a greater abundance and looser arrangement of mesophyll cells.

Spot tests with diphenylamine-sulphuric acid showed that there was a consistent above-normal accumulation of nitrates in the leaf tissue of molybdenum-deficient cauliflower plants. This finding is in accord with that of other workers (9), and has been found to be a useful indication of incipient molybdenum deficiency in the field. It was noted, however, that an accumulation of nitrates could occur in the absence of whiptail symptoms.

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In several areas where whiptail was found, nearby potato plantings were affected with a stem-streak disorder caused by an excess of manganese. In such locations cauliflower plants often developed a distinct upward curling of leaf margins, a symptom that has been associated with manganese toxicity (4). Manganese has been shown to have an inhibiting effect on uptake of molybdenum (4), and this physiological relationship between the two nutrients may be a contributing factor in the commonly observed coincidence of manganese toxicity and molybdenum deficiency in Prince Edward Island.

CONTROL OF WHIPTAIL

Whiptail is endemic in southern England and in parts of Australia. It has been prevented in both countries by heavy applications of ground limestone or by adding sodium molybdate to the fertilizer at rates varying from 1 to 10 lb. per acre (5, 7). Applications of molybdate salts, in solution, to the seedbed and to foliage have been recommended (6, 7). In Holland excellent control was obtained by spraying foliage with 0.01 to 0.1 per cent ammonium molybdate (8), and in Florida by a spray applied at the rate of 0.35 lb. of the salt per acre (2).

Several soil amendments and methods of molybdenum application have been tried for the prevention and correction of whiptail in Prince Edward Island in areas where the disease had previously occurred.

Effect of Some Soil Amendments

The comparative effects of lime, barnyard manure and sodium molybdate applications were tested by applying these amendments to the soil in early spring before the cauliflower transplants were set out. Two varieties were used, one of them from two seed sources. Deficiency symptoms began to appear during midsummer, one month after trans-

TABLE 1.—THE EFFECT OF SEVERAL SOIL AMENDMENTS ON THE DEVELOPMENT OF WHIPTAIL IN CAULIFLOWER

Soil amendment[2]	Percentage of plants showing whiptail[1]		
	Variety		
	Ideal	Super-Snowball Seed Source A	Super-Snowball Seed Source B
Limestone at 3000[3] lb. per acre	41.7	0	25.0
Sodium molybdate at 10 lb. per acre	0	0	0
Limestone and sodium molybdate at above rates	0	0	0
Barnyard manure at 15 tons per acre	16.6	4.1	16.6
Check	58.3	4.1	62.5

[1] There was a total of 360 plants in this split-plot randomized block experiment comprising 3 varieties, 5 treatments and 3 replicates.
[2] All plots received 5-10-13 fertilizer at 1 ton per acre.
[3] Liming raised soil pH from 4.88 to 6.15.

FIGURE 1. Whiptail in the variety Super-Snowball and its prevention by foliar application of molybdenum.
Left: No molybdenum applied.
Right: Molybdenum sprayed on foliage at the rate of 0.2 lb. ammonium molybdate per acre when plants were in the 3-leaf stage. Both plants 10 weeks old.

TABLE 2.—THE EFFECT OF SOIL APPLICATIONS OF SODIUM MOLYBDATE ON WHIPTAIL DEVELOPMENT IN SUPER-SNOWBALL CAULIFLOWER

Sodium molybdate application (lb. per acre)	Percentage of whiptail plants[1]	
	Seed Source A	Seed Source B
0	6.5	21.3
2	0.9	4.4
8	0.9	0.0

[1] There was a total of 602 plants in the split-plot Latin square experiment, or an average of 33.4 plants per sub-plot.

planting. Treatment effects were evaluated in early autumn by counting the plants that showed typical whiptail. The treatments and their effects on varieties are given in Table 1. The results show that sodium molybdate at the rate of 10 lb. per acre was completely effective in correcting the deficiency. Heavy applications of limestone or manure gave only partial control. A comparison of varieties showed that Ideal was very susceptible to whiptail and that Super-Snowball from one seed source was affected less by it though not necessarily more resistant than the same variety from another source.

The effectiveness of reduced application rates of molybdenum was tested in another market garden by using two seed sources of the variety Super-Snowball. Ten days before transplants were set out this area received molybdenum applied in a 5-10-13 fertilizer mixture broadcast at the rate of 1 ton per acre. The results of the molybdenum treatments on whiptail development (Table 2) show that an application of 8 lb. per acre was almost completely effective in preventing whiptail symptoms. Once again, a marked difference was evident in the susceptibility of plants from the two seed sources.

Effective control was achieved in several other instances by field applications of sodium molybdate before transplanting. In one, where check plots showed 6 per cent whiptail, a broadcast application of 1 lb. per acre of the salt prevented symptom development; in a second area, where check plots showed 50 per cent whiptail, an application of 4 lb. of the salt per acre corrected the deficiency.

Effect of Foliar Applications of Molybdate Salts

Foliar sprays were tested in two plantings which presented a chlorotic, unthrifty appearance while still in the 3-leaf stage. Tissue analysis also showed a marked accumulation of nitrates in the leaves. In one of these plantings two sprays were applied, the first during the 3-leaf stage, the second when the plants were half-grown. Each spray delivered sodium molybdate at the rate of 1 lb. per acre. After a period of 6 weeks, a striking difference was apparent between sprayed and unsprayed plants (Figure 1). By heading time the unsprayed check rows had developed whiptail in 90 per cent of the plants, whereas the treated plants were completely free of symptoms and were heading normally. In a second planting, where 40

per cent of the plants later became affected, a single application of ammonium molybdate at the rate of 0.2 lb. per acre while the plants were in the 3-leaf stage was effective in correcting the deficiency.

DISCUSSION

The symptoms described for molybdenum deficiency in cauliflower and the effectiveness of molybdate salts for its correction are in general agreement with other reports in the literature. All cases under observation occurred on acid, sandy-loam soils to which little manure or lime had been applied and where manganese was often in excess.

Both varieties used in the tests were subject to whiptail but Super-Snowball plants from one source of seed were affected much less severely. It is probable that this seed had a high molybdenum content. Waring *et al.* (7) emphasize the importance of producing seed on land where minor elements are in good supply.

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NOTE ON THE USE OF 8-QUINOLINOL IN CHLOROFORM FOR CLEANING MINERAL GRAINS FROM SOILS FOR IDENTIFICATION

Moeller (1) used a solution of 8-quinolinol in chloroform to extract various metal ions, including ferric ion, from aqueous solution. He estimated the amounts of the metals so extracted from the light absorption of the compounds formed. More recently it was reported (2) that the iron contained in an aqueous suspension of ferric hydroxide could be extracted similarly. The present communication presents evidence of the utility of this type of extraction for the removal of iron-containing stains from the surface of mineral grains from the sand fraction of soils in preparation for identification of the minerals under the petrographic microscope.

25 ml. of 0.1M 8-quinolinol solution in chloroform and 1 ml. of distilled water were added to 1-gm. samples of soil mineral grains in separatory funnels which were then placed on a rotatory shaker and allowed to shake continually for 24 hours. The reaction would not take place unless water was present in small amounts. Replacement of the chloroform-quinolinol phase at the end of each 24-hour period gave more rapid cleaning. On completion of the third 24-hour period the soil mineral grains were found to be free of iron-containing stains. However, they had a greenish tinge which interfered with petrographic identification but which was easily removed with dilute acetic acid. The period of contact with the reagent to remove stains having the appearance of iron oxide has not, so far as the writer has been able to determine by subsequent examination under the petrographic microscope, resulted in appreciable attack upon minerals of specific gravity less than 2·95. Minerals of specific gravity greater than 2.95 are at present under study.

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